one plug each, all containing sperm, and 70 showed positive penile smears. A number of hooded Long-Evans rats, observed in a less systematic way, gave similar results.

These figures for the incidence of seminal plugs are probably conservative. Many plugs must go unrecognized because of their small size. Some are no bigger than the head of a pin, and are yellow and translucent, while others are as large as a kernel of corn. Sometimes a plug is missed because of its flat shape (probably fashioned by lying against the side of the ensheathed penis).

Spontaneous ejaculations first appear in hooded rats when they are between 50 and 60 days old. Sperm is often found in these first plugs and in the penile smears at the same age. Rats 16 months old, our oldest, continue to ejaculate spontaneously.

Occasionally a rat will deposit seminal plugs repeatedly, day after day for weeks. When paired with a female, such a male will continue to ejaculate without intromission (the vaginal smears of the cage mate reveal an absence of cornified cells and sperm, thus ruling out intromission).

After castration of rats, the frequency of ejaculation tapers and ceases within 7 to 10 days. (Sperm continues to appear in the initial postcastration plugs.) Conversely, a daily injection of testosterone propionate (0.5 mg) seems to reduce the incidence of seminal plugs. These results are contrary to the known androgenic control of male sexuality. However, there is little evidence on the hormonal control of grooming, and I am inclined to argue that the level of testosterone may determine the extent to which the rat will groom the genital region orally. This may explain why the rat with abnormally small testes (No. 16) seemed to produce the most semen, when unrestrained.

As regards autonomic involvement (*5*), seminal plugs were found within 30 min after the injection of epinephrine (0.07 mg subcutaneously), and within 4 min after the injection of mecholyl (5 to 8 mg subcutaneously). Under neither condition was an erection seen.

In order to rule out visual and olfactory cues in eliciting the seminal flow, we have redistributed rats in cages and rearranged cages in the rack with no effect on spontaneous ejaculation.

We have investigated other species but have not indentified sperm in the smears of any of them. Our samples included mice, rabbits, cats, and monkeys. Only the mice, when restrained from grooming the genital region orally, deposited seminal plugs.

We cannot distinguish the spontaneously produced plugs from those produced during copulation. This raises the issue whether fertile copulation need involve a *triggered* ejaculation following a series of intromissions. The multiple intromissions may serve the function of maintaining genital contact until a *spontaneous* ejaculation takes place. Alternatively, the spontaneous seminal discharges may represent an overflow of seminal fluid. In this sense, it may be possible to distinguish between "ejaculation" and "discharge" (*6*).

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14 August 1961

Immunization to Schistosoma mansoni in Mice Inoculated with Radiated Cercariae

Abstract. Preliminary experiments indicate that mice inoculated with cercariae of *Schistosoma mansoni* that have been exposed to cobalt-60 radiation in the range of 2500 to 3000 rep develop immunity to reinfection with nonradiated cercariae.

Resistance in mice and monkeys to reinfection with *Schistosoma mansoni* has been demonstrated by several investigators (*1*). Other reported studies include attempts to induce immunity by the inoculation of animals with immune serum, use of killed worms, and injection with extracts or metabolites of the worms (*2*). Instead of employing killed worms or metabolic products of *S. mansoni,* we thought that immunity might be induced by inoculation of cercariae exposed to an amount of radiation which would produce sexual sterilization and yet allow the cercariae to migrate in the tissues of the host in order to exert an antigenic stimulus.

Previous investigators have demonstrated that animals will develop immunity to reinfection with certain helminths after a primary infection with radiated larvae [*Trichinella spiralis* (*3*), *Dictyocaulus viviparus* (*4*), *Haemonchus contortus* (*4*), *Ascaris suum* (*5*)].

In the present investigation, cercariae from the Puerto Rican strain of the snail, *Australorbis glabratus,* were employed. In the initial experiment, 22 Swiss mice were divided into five groups: four groups of four mice each and one group of six mice. Each animal was inoculated intraperitoneally with 300 cercariae of mixed sexes. The first group received nonradiated cercariae. The second group received cercariae exposed to 1000 rep (roentgen equivalent physical); the third group, cercariae exposed to 2500 rep; the fourth group, cercariae exposed to 5000 rep; and the fifth group, cercariae exposed to 7500 rep. Eight weeks after the inoculation all the mice were killed and their livers and mesenteric veins were examined for schistosomes according to the method of Yolles *et al.* (*6*).

In a second experiment, five like groups of mice were similarly inoculated with nonradiated and radiated cercariae. After 8 weeks, each animal was given a challenging infection of 300 nonradiated cercariae of mixed sexes. The animals were then killed and examined for schistosomes 4 weeks after the challenging infection. At the time the inoculated mice were challenged, members of a sixth group of uninoculated mice each received 300 of the nonradiated cercariae.

In the first experiment, the mean (geometric) number of schistosomes per mouse recovered from each group receiving cercariae radiated with 0, 1000, 2500, 5000, and 7500 rep was 51.5, 29.0, 1.5, 0, and 0, respectively (Fig. 1, top). In the second experiment, the mean number of schistosomes recovered per mouse, after challenge, from groups first inoculated with cercariae radiated with 0, 1000, 2500, 5000, or 7500 rep was 46.4, 33.8, 6.6, 27.3, and 38.2, respectively. The mean

Table 1. Number of schistosomes recovered from mice, each of which was initially immunized with one or two doses of 200 radiated cercariae and later challenged with 200 nonradiated cercariae.

Group	No. of mice	First inoculation: radiation dose (rep) to cercariae	Time schedule (weeks after first inoculation)			Average No. of worms recovered
			Second inoculation (3000 rep to cercariae)	Challenge with nonradiated cercariae	Mice killed	
A	5	0			7½	59.6
B-1	5	3000			8	0.8
B-2	5	3000		10	18	8.0
C-1	5	3000	7½		15½	0.4
C-2	5	3000	7½	13½	21	5.2

number of schistosomes recovered from the uninoculated mice that received only the challenge infection of 300 nonradiated cercariae was 58.0 (Fig. 1, bottom).

The first series of tests located the radiation doses applied to cercariae which would reduce or eliminate the yield of adult worms. Compared with the yield when no radiation was used, the mean number of worms was smaller at 1000 rep, was drastically reduced at 2500 rep, and was zero at 5000 rep and 7500 rep.

The second series tested the effectiveness of the inoculation in inducing immunity. The mean number of worms recovered from mice given a challenging infection showed that prior inoculation with cercariae exposed to 7500 rep was ineffective, while cercariae exposed to 5000 rep produced a small measure of immunity. Cercariae exposed to 2500 rep produced a marked immunity; the mean number of worms recovered from mice immunized with cercariae exposed to 2500 rep was only 6.6, compared to 58 for the nonimmunized controls. The number of worms recovered from mice which were challenged after receiving nonradiated cercariae or cercariae exposed to 1000 rep was high. However, we cannot say how many worms were the result of the first inoculation and how many were produced from the challenge.

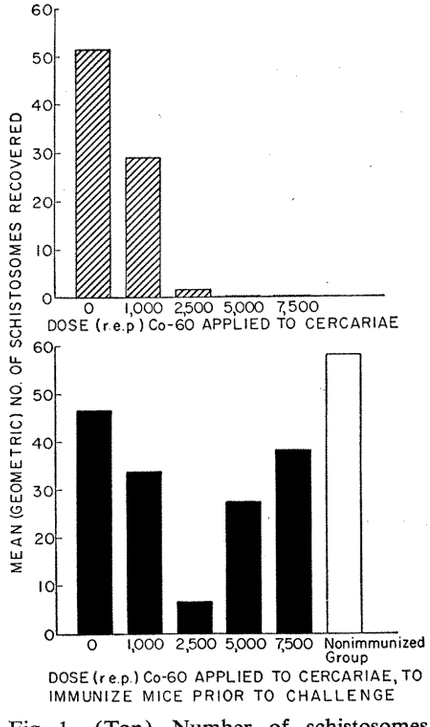

Fig. 1. (Top) Number of schistosomes recovered from mice 8 weeks after each animal was inoculated with 300 cercariae radiated with designated dose of cobalt-60. (Bottom) Number of schistosomes recovered from immunized mice 4 weeks after each animal was challenged with 300 nonradiated cercariae. Each mouse had been inoculated 8 weeks prior to challenge with 300 radiated cercariae with designated doses of cobalt-60.

In a third experiment, the effect of two inoculations of radiated cercariae was compared with that of one inoculation of radiated cercariae. The cercariae used in the inoculations had been exposed to 3000 rep. As before, nonradiated cercariae were employed for control experiments and to challenge the inoculated animals. Groups of five mice each were used. Mice in Group A were used as control animals which were inoculated only with 200 nonradiated cercariae; mice in group B-1 received one inoculation of 200 radiated cercariae each; mice of group C-1 received two such inoculations each, the second given 7½ weeks after the first.

The mice in group B-2 received one inoculation and those in C-2 two inoculations of radiated cercariae, and later were challenged with 200 nonradiated cercariae (Table 1).

In groups B-1 and C-1, single or double inoculations with radiated cercariae produced negligible average numbers of worms (0.8 and 0.4, respectively). When challenged with 200 nonradiated cercariae, the average number of worms from the mice inoculated once (B-2) was 8.0 and from those inoculated twice (C-2) was 5.2. The average number of worms recovered from the control group (A), inoculated only with nonradiated cercariae, was 59.6.

Examination of the few worms that developed from cercariae that received 3000 rep cobalt-60 showed that they were sexually sterile. Therefore, the inoculation of cercariae exposed to 3000 rep did not appear to produce an infection.

Examination of microscopic sections of the livers of animals which had received one or two inoculations of radiated cercariae, followed by a challenging infection with nonradiated cercariae, revealed no more than four eggs and granulomata in any section of liver. The control animals which received nonradiated cercariae yielded up to 50 eggs and granulomata per section of liver. Eleven microscopic sections of the small intestines of animals which received only the radiated cercariae showed one granuloma with an egg in only one animal, whereas in controls, 15 and 24 eggs and granulomata, respectively, were seen in two sections examined.

The results of this study indicate that mice inoculated with cercariae of *S. mansoni* which have been exposed to cobalt-60 radiation in the range of 2500 to 3000 rep develop immunity to reinfection with nonradiated cercariae.

Further experiments are being carried out with radiated cercariae to determine optimal conditions, such as dose of radiation, number of cercariae to be inoculated, and intervals of immunizing doses, for production of maximal degrees of immunity (7).

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Senescence Inhibition and Respiration

Abstract. Freshly harvested asparagus spears treated with N^6-benzyladenine and then held in the dark at 21°C for 136 hours showed a lower respiration rate, as measured by CO_2 evolution, than nontreated spears. Associated with the lower respiration rate were proportional decreases in postharvest spear elongation and weight loss through desiccation.

Since the discovery of the biological activity of kinetin (6-furfurylaminopurine) in cell division (*1*), many studies have been made in which this and similar compounds have been evaluated in cell division of tobacco tissue, germination of lettuce seeds, and cell enlargement in leaf disks of radish (*2*). In addition, some rather striking effects on protein metabolism, chlorophyll breakdown, and the capacity of excised leaves to withstand stress have been reported (*3*). N^6-benzyladenine, a compound in which the furan ring in kinetin has been replaced by a benzene ring, has shown some effect in preventing postharvest breakdown of head lettuce when applied shortly before harvest or as a postharvest dip (*4*). This compound reportedly has a similar effect on other green vegetables, including asparagus (*5*).

Freshly harvested asparagus spears were obtained from a commercial processor and held for 24 hours at 5°C; the apical ends were trimmed to 5-inch length and then dipped in a $5 \times 10^{-5} M$ solution of N^6-benzyladenine or water. Eight 500-g samples of the treated spears and like samples of nontreated spears were placed in respirometers (*6*) and held in the dark at 21°C. The CO_2 evolution was measured every 8 hours for 136 hours, whereupon the spears were removed and their weight loss and length were determined.

The observed mean values and the fitted regression lines describing CO_2 evolution in treated and nontreated asparagus spears (Fig. 1) indicate that treatment with N^6-benzyladenine materially decreased respiration. Theoretical values of total CO_2 evolution during the 136-hour interval, obtained by integration of the regression equations, were 303 mg/kg for the nontreated and 257 mg/kg for the treated spears. These calculated values correspond very closely to the observed values of 295 and 253 mg/kg. The area between the curves constitutes the total respiration inhibition. This calculation yielded a value of 48 mg of CO_2 per kilogram, indicating about a 16-percent reduction in respiration through the first 120 hours in the N^6-benzyladenine-treated samples. Observed values for these differences were 47 mg of CO_2 per kilogram and 16 percent, respectively. After 120 hours, the nontreated samples evolved CO_2 at a lesser rate than did the treated, as a probable consequence of an initially more rapid depletion of the metabolites in the nontreated samples.

Weight losses in 136 hours were 7.25 and 5.95 percent in the nontreated and treated samples, respectively. Though the differences in weight loss were largely a function of the degree of desiccation, it is noteworthy that the decrease in water loss of approximately 18 percent in the treated samples was comparable to the decrease in respiration. Furthermore, nontreated spears elongated an average of 8.2 percent, and treated spears, of 7.0 percent. This difference (15 percent less elongation in the N^6-benzyladenine-treated spears) was again directly proportional to the amount of respiration inhibition.

These data suggest that respiration as measured by CO_2 evolution, desiccation as measured by weight loss, and growth as measured by elongation are all proportionally inhibited in harvested asparagus spears following postharvest application of N^6-benzyladenine.

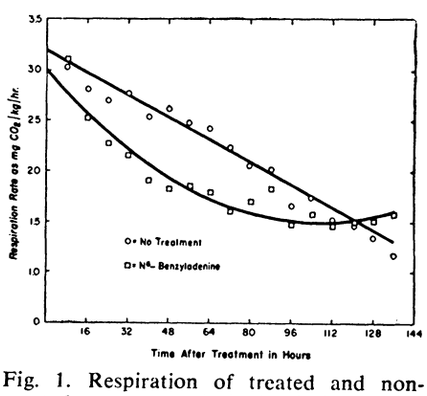

Fig. 1. Respiration of treated and nontreated asparagus held in the dark at 21°C.

It is further suggested that many of the phenomena observed by others, such as chlorophyll retention, changes in nitrogen metabolism, and general inhibition of senescence in green plant tissues subsequent to treatment with kinetin and related compounds, may be a consequence of respiration inhibition of the type described in this report (*7*).

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3 July 1961

Explanation of Cocaine Desensitization of Blood Pressure Responses to Ephedrine

Abstract. It is suggested that cocaine desensitizes ephedrine blood pressure responses by depletion of norepinephrine depots. This was shown by reversing cocaine desensitization through the administration of agents such as bretylium, which has a sparing effect on norepinephrine, and by the infusion of norepinephrine itself.

A number of workers (*1*), since the original discovery by Tainter (*2*), have shown that cocaine in large doses diminishes or abolishes the pressor responses to ephedrine. The exact mechanism of this antagonism has not been explained. We therefore attempted in the present study (*3*) to elucidate this phenomenon according to the theory of one of us (T.K.). This theory postulates that large doses of cocaine exhaust the sympathetic neurohumor depots and, owing to this mobilization, may change physicochemical parameters of cellular membranes (*4*). Therefore, three alternatives were considered: ephedrine should be protected from cocaine desensitization by the administration of (i) substances which prevent such neurohumoral release, or (ii) substances which protect the neurohumor from metabolic destruction, or (iii) the neurohumor itself.

Male mongrel dogs, weighing from 6 to 10 kg, were anesthetized intraven-