creased to reach the level of untreated cells at 72 hours. This temporal decline in the frequency of aberrant cells may be due to reproductive death of the abnormal cells consequent to chromosomal damage.

These experiments demonstrate that cultured fibroblasts from NZB fetuses manifest increased sensitivity to ultraviolet radiation. The findings are similar to the chromosomal instability reported in cells cultured from patients with xeroderma pigmentosum (*3*). We do not know the mechanism underlying the chromosomal fragility induced by ultraviolet radiation in NZB fetal fibroblasts. It may be relatively specific to ultraviolet radiation, being a result of defective DNA repair or activation of latent viruses which then cause chromosomal abnormalities. Alternatively, NZB chromosomes may be generally unstable and exhibit fragility upon exposure to many noxious agents.

All of the inherited chromosomal instability syndromes in man are accompanied by increased frequency of malignancy (*1*). Similarly, most aging NZB mice develop lymphoreticular malignancy of the spleen and other organs (*5*). These animals also have clones of aneuploid spleen cells with specific karyotypic changes, especially trisomy 15 (*10*). We do not know how the chromosomal instability in NZB fetuses is related to the aneuploid clones and lymphoreticular malignancy characteristic of adult animals of this strain. However, it is tempting to suggest that the chromosomal instability favors the development of specific chromosomal changes which in turn play a role in the evolution of neoplasia.

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References and Notes

1. J. German, *Prog. Med. Genet.* **8**, 61 (1972); T. M. Schroeder, in *Chemical and Viral Oncogenesis*, P. Bucalossi, U. Veronesi, N. Cascinelli, Eds. (Elsevier, New York, 1975), p. 62; F. Hecht and B. K. McCaw, in *Genetics of Human Cancer*, J. J. Mulvihill, R. W. Miller, J. F. Fraumeni, Jr., Eds. (Raven, New York, 1977), p. 105.
2. J. E. Cleaver, *Nature (London)* **218**, 652 (1968).
3. J. M. Parrington, J. D. Delhanty, H. P. Baden, *Ann. Hum. Genet.* **35**, 149 (1971); M. S. Sasaki, *Mutat. Res.* **20**, 291 (1973); A. D. Schonwald and E. Passarge, *Hum. Genet.* **36**, 213 (1977); S. Wolff, B. Rodin, J. C. Cleaver, *Nature (London)* **265**, 347 (1977).
4. J. B. Howie and B. J. Helyer, *Adv. Immunol.* **9**, 215 (1968).
5. J. East, *Prog. Exp. Tumor Res.* **13**, 84 (1970).
6. M. Bieschowsky and F. Bieschowsky, *Nature (London)* **194**, 692 (1962).
7. B. Halpern, I. Emerit, E. Housset, J. Feingold, *ibid.* **253**, 214 (1972).
8. P. J. Fialkow, G. R. Paton, J. East, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1094 (1973).
9. The NZB/Sea colony was originally obtained at the 95th generation of inbreeding from the University of Otago through the courtesy of C. M. Goodall and has been maintained in Seattle for an additional 12 generations. Primary cultures of fetal fibroblasts from NZB and BALB/c mice were initiated from minced fragments of at least three to four pooled embryos obtained from mice on days 14 to 18 of pregnancy. The cells were grown in 25 cm^2 Falcon flasks containing RPMI-1640 supplemented with 15 percent heat-inactivated fetal calf serum (Gibco), 100 units of penicillin-streptomycin mixture, 25 m*M* Hepes buffer, and $NaHCO_3$ (2 g/liter; RPMI-tissue culture medium). The cultures were incubated at 37°C in a humidified atmosphere with 5 percent CO_2. Cultures were tested at intervals and found to be negative with the agar technique for mycoplasma contamination. Primary or single-passaged cultures were used in all experiments. The duration of the cell cycle was similar in NZB and BALB/c cultures (26 ± 4 hours). When the cells were semiconfluent they were harvested by briefly dissociating them with 0.05 percent trypsin (Difco, 1:250) in Vernal buffer, and 2×10^5 cells were replated in 35-mm Falcon dishes containing 1.6 ml of RPMI-tissue culture medium. The cells were grown for 24 hours, washed twice with Tyrode's solution, and then immediately irradiated with ultraviolet light at a dose rate of 1 J/mm^2 sec^{-1} at a peak wavelength at 254 nm with an ultraviolet lamp (Ultraviolet Products) without any overlying medium in the dish. Controls were treated similarly, but were not exposed to ultraviolet radiation (in the absence of ultraviolet light, this procedure itself does not affect the prevalence of chromosome abnormalities). After the cells were irradiated, 3 ml of RPMI-tissue culture medium was added to each dish and the cells were incubated for another 24 hours. For cytogenetic analysis, 0.02 ml of colchicine (20 mg/ml) was added 2 hours prior to harvesting. Trypsinized cells were treated with hypotonic KCl (0.075*M*) for 15 minutes at room temperature and then fixed overnight in a mixture of acetic acid and methanol (1:3 by volume). The cells were stained with Giemsa for cytological study. Well-spread metaphases (60 to 100) selected randomly were scored on each slide at × 1000 in oil immersion without the experimenter having knowledge of the cells' origins.
10. P. J. Fialkow, J. I. Bryant, J. M. Friedman, *Int. J. Cancer* **21**, 505 (1978).
11. This work was supported by the Medical Research Service of the Veterans Administration and grant GM 15253 from the Institute of General Medical Sciences, National Institutes of Health.

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Histidine-Rich Protein as a Model Malaria Vaccine

Abstract. *Ducklings were successfully immunized against* Plasmodium lophurae *with a purified and characterized histidine-rich protein as antigen. The use of adjuvant is not required for this protective effect, and immunity can be passively transferred with serum.*

As vector control and chemotherapy have not proved sufficient measures for curbing malaria, which affects 100 million humans yearly, attention has been turned to additional alternative methods of control, including the possibility of vaccination (*1*). Although successful vaccinations in various animals and even in humans have been reported (*2*), two major difficulties have made the feasibility of extrapolating the existing methods for mass human vaccination impractical: (i) the unavailability of sufficient amounts of suitable antigens and (ii) contamination of antigens with host components that could raise problems of eliciting autoimmune response.

I now report the successful immunization of ducks against malaria with a purified and characterized protein. The protein (HRP) was isolated from the avian malaria *Plasmodium lophurae* grown in ducklings. It was found to have a rather unusual composition, with about 70 per-

Table 1. Pattern of infection in immunized and control ducks. Ducks 33 to 37 were injected (intramuscularly) with an emulsion of histidine-rich protein and Freund's complete and incomplete adjuvants (day 0) and received two intravenous booster injections of antigen alone (days 4 and 7). Ducks 170 to 173 were treated as the group above except for substitution of phosphate-buffered saline for antigen. Ducks 51 to 54 were not treated. All groups were challenged on day 14 after immunization was started with 2.6×10^8 parasitized red blood cells (RBC).

Duck No.	Percent infected RBC after challenge						
	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
33	3	6	20	40	33	20	Died
34	5	12	12	18	35	38	Died
35	3	5	25	44	45	74	Died
36	2	4	1	0.1	0	0	0
37	4	10	30	59	39	Died	
170	4	10	50	86	Died		
171	5	23	68	94	Died		
172	4	35	62	92	Died		
173	3	25	70	95	Died		
51	4	20	58	Died			
52	3	27	50	90	Died		
53	0.5	4	25	47	90	Died	
54	2	20	52	95	Died		

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cent histidine and four other major constituent amino acids: proline, alanine, glutamic acid, and aspartic acid (*3*). Since indirect experimental evidence indicated that the protein could have a role in the penetration of merozoites into erythrocytes (*4, 5*), it seemed reasonable to test it as a vaccine.

The protein was prepared as an acid extract as described (*3*). To remove residual heme that usually binds to the protein, the extracts were further purified by filtration through a Sephadex G-100 column in 50 percent acetic acid. Eluates were dialyzed in distilled water, and the protein concentration was determined by a micro biuret method (*6*). As the protein is insoluble at physiological *p*H, measured amounts were precipitated in phosphate-buffered saline, *p*H 7.4, and injected as insoluble suspensions. The reported antigen concentrations are as accurate as can be expected from measurements of suspensions.

Trial experiments were performed to define proper conditions for successful immunization, such as age of host, schedule of immunization, antigen dose, and route of inoculation. Even though birds become immunologically mature at about 6 weeks of age, immunization had to be initiated when ducklings were 4 to $4^1/_2$ weeks old, and a massive dose of challenge inoculum had to be given within 15 days since some ducklings, by 8 to 9 weeks of age, develop natural resistance to the infection. In the first successful experiment, two out of three immunized ducks survived the challenge inoculation. The experiment was repeated with a larger group of animals and appropriate controls. On day 0, $4^1/_2$-week-old ducklings were injected intramuscularly with an emulsion of 1 mg of HRP in 0.5 ml of phosphate-buffered saline, 0.25 ml of Freund's complete adjuvant, and 0.25 ml of Freund's incomplete adjuvant. On days 4 and 7, the immunized ducks received intravenously 0.7 mg and 1 mg of antigen, respectively. One group of control ducklings was treated in the same manner except that the phosphate-buffered saline was substituted for antigen, while a second group was not treated. On day 14, all birds were challenged (Table 1); all control ducks died 8 days after challenge, one immunized duckling showed full recovery, and four had lower levels of parasitemia and 2 days extended life.

Because of these encouraging results, the experiment was repeated with some modifications. While the schedule of immunization and route of inoculation were kept the same, the amount of antigen was increased and the challenge dose was decreased. Two additional groups of experimental ducks were also included to test (i) whether the use of adjuvant was required for the protective immune response and (ii) whether commercially available synthetic polyhistidine (Sigma; molecular weight, 5000 to 8000) could be substituted for the histidine-rich antigen. The four groups of birds were injected as follows. At day 0 (intramuscularly) group A was given 2 mg of HRP in 1 ml of phosphate-buffered saline plus 0.25 ml of Freund's complete adjuvant and 0.75 ml of Freund's incomplete adjuvant; group B was given 2 mg of HRP in phosphate-buffered saline; group C was given 2 mg of polyhistidine plus adjuvants as described for group A; and group D was given adjuvant plus phosphate-buffered saline. On day 4 (intravenously) group A was given 1 mg of HRP; group B was given 1 mg of HRP; and group C was given 1 mg of polyhistidine. On day 7 (intravenously) group A was given 1 mg of HRP; group B was given 2 mg of HRP; and group C was given 2 mg of polyhistidine. On day 14 all birds were challenged (Table 2). All ducks immunized with HRP and adjuvant (group A) survived; three of these showed very low parasitemia on day 5 after challenge and no parasites were detectable by day 7 after challenge. The fourth duckling (No. 62) showed a peak parasitemia of 35 percent, but this peak was followed by the appearance of degenerate parasites and the gradual decline of the number of infected erythrocytes. The duckling was very anemic but showed full recovery by day 17. Three out of four ducks in group B survived challenge, indicating that HRP could elicit a protective immune response without the aid of adjuvant. Polyhistidine was completely ineffective, and all ducklings in group C died about the same time as the controls (group D).

Table 2. Pattern of infection in immunized and control ducks. Group A was injected (intramuscularly) with histidine-rich protein emulsified with Freund's complete and incomplete adjuvants (day 0) and received two intravenous booster injections of antigen alone (days 4 and 7). Group B was treated as was group A except that adjuvant was excluded. Group C was immunized with polyhistidine (5000 to 8000 molecular weight) with the inclusion of adjuvant as described for group A. Group D received (intramuscularly) adjuvant emulsified with phosphate-buffered saline on day 0 and was given no further treatment. All groups were challenged on day 14 with 8.5×10^7 parasitized red blood cells (RBC).

Duck No.	Percent infected RBC after challenge				
	Day 5	Day 7	Day 9	Day 10	Day 11
		Group A			
62	4	35	20		20*
63	2	0	0		0
64	2	0	0		0
65	0.5	0	0		0
		Group B			
76	4	44	91	Died	
77	2	0	0		0
78	0.1	0	0		0
79	1	0	0		0
		Group C			
69		24	79	Died	
70		23	95	Died	
22		18	62		Died
23		28	90	Died	
71		42	Died		
		Group D			
80	3	10	39		Died
81	2	16	74	Died	
192	2	26	95	Died	
193	4	30	90	Died	

*Duck 62 showed 15 percent parasitemia by day 14 and 0.5 percent by day 16. No parasites were detectable on day 17.

Passive transfer of immunity to malaria by serum of convalescing subjects has been demonstrated in several species (*2*). Serum was collected from duck No. 36 (Table 1) 2 weeks after challenge, and a crude globulin fraction was prepared from it by precipitation with sodium sulfate. Three 8-day-old ducklings were each injected with the globulin fraction obtained from 5 to 6 ml of serum; they were then challenged with 9×10^6 parasitized erythrocytes. All three ducklings survived although there was variation in the course of infection; one duckling showed a peak parasitemia of 1 percent, a second showed 28 percent, and the third showed 36 percent before recovery. Control birds injected with equivalent amounts of normal globulin showed the expected course of infection and died. When passively immunized ducklings were challenged 2 weeks and 4 weeks after recovery they showed total resistance. This is quite different from ducks made immune by infection and drug cure; it requires three to four infections followed by chloroquine treatment to render a duck resistant to reinfection with *P. lophurae*. Globulin fractions collected from serums of immunized ducklings prior to challenge also showed protection of young ducklings. These serums have given less consistent results; five serums were protective out of 12 trials. It is possible that 7 days after the last booster of antigen when serums were collected the antibody titer was not at its peak. In addition, there may be other elements of the immune response that aid in controlling the infection within the immunized host.

Viral infections in ducklings that may interfere with normal development of *P. lophurae* in this host have been reported (*7*). In my laboratory I have never had a

duckling survive an infection with the strain of *P. lophurae* used here. Occasionally, typical symptoms of the spleen necrosis virus have been observed. This agent can be transmitted to healthy ducklings by inoculation of small volumes of plasma; even though this virus does slow down normal development of *P. lophurae*, it also invariably kills infected ducklings (*7*). The possibility that the observed protection was due to any other infectious agent transferred with immune globulin was eliminated experimentally. Six 8-day-old ducklings were each inoculated with 0.5 ml of plasma from each of six immunized ducks. When challenged with an infective dose of *P. lophurae*, all six ducklings showed the expected course of parasitemia and died. However, globulin fractions of serums from three of the same six immunized donor ducks protected recipient ducklings from *P. lophurae* infection. When plasma (0.5 ml) from each of the three protected ducklings was inoculated into three other ducklings, the course of infection with *P. lophurae* was not altered.

The HRP from *P. lophurae* is the only chemically characterized protein from malaria parasites. A similar protein was demonstrated in *P. falciparum* (*8*), but the possible immunological cross-reactivity of the proteins from these two species remains to be determined. The finding of cross-reactivity could lead to a possible synthetic malaria vaccine. Even though through the use of crude antigens it has been concluded that protection against malaria is species-specific, the possibility of immunological cross-reactivity to a purified antigen cannot be ruled out without experimental evidence. In addition, the rather peculiar amino acid composition of HRP could lend itself to immunological alteration through chemical manipulation.

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References and Notes

1. "Developments in Malaria Immunology," *WHO Tech. Rep. Ser.* (No. 579) (1975).
2. A. Zuckerman, *Exp. Parasitol.* **42**, 374 (1977).
3. A. Kilejian, *J. Biol. Chem.* **249**, 4650 (1974).
4. ———, *J. Protozool.* **23**, 272 (1976).
5. ———, in *Biochemistry of Parasites and Host-Parasite Relationships*, H. van den Bossche, Ed. (North-Holland, Amsterdam, 1976), p. 441.
6. R. F. Itzhaki and D. M. Gill, *Anal. Biochem.* **9**, 401 (1964).
7. W. Trager, *Proc. Soc. Exp. Biol. Med.* **101**, 578 (1959).
8. A. Kilejian and J. B. Jensen, *Bull. WHO* **55**, 191 (1977).
9. Supported mainly by AID contract ta-C-1429 and in part by NIH grant AI-13728. I thank E. Kracauer and M. Thomassen for technical assistance.

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Schistosoma mansoni: Identification of Chemicals That Attract or Trap Its Snail Vector, *Biomphalaria glabrata*

Abstract. *A new bioassay for chemical attractants of aquatic snails demonstrated that* Biomphalaria glabrata *could be attracted to or trapped in the vicinity of homogenates of lettuce. Fractionation of homogenates revealed the amino acids glutamate and proline as the primary attractants. Attraction was specific for the* L *form of glutamate. Proline appeared to stimulate reproductive activity. Glutathione, γ-aminobutyric acid, and a number of other compounds had no effect. Extracts of lyophilized snail tissue also attracted other snails and may thus contain pheromones. These results permit formulation and testing of controlled-release attractants designed to overcome the repellant effects of slow-release molluscicides, as well as the design of stimulants to be used with no-release poisons.*

Molluscicides targeted at snail vectors of important human pathogens such as schistosomes frequently repel the snail from areas containing lethal doses (*1*). The advent and success of slow- or controlled-release technology for dispensing pesticides has provided new and exciting possibilities for developing baits, attractants, or other chemostimulants which will enhance the efficacy of these formulations (*2*). Attraction or trapping of *Biomphalaria glabrata*, the snail host for *Schistosoma mansoni*, by various substances, especially food sources, has been reported (*2–5*). But specific chemicals emanating from food sources or the snails themselves, which can attract or trap the snail, have not been identified. We report herein the isolation and identification of chemicals from a food source and from snails which attract or trap *B. glabrata*. Fractionation of lettuce revealed that the portion containing free amino acids was the primary stimulant.

Table 1. Responses of *B. glabrata* to Romaine lettuce homogenate and fractions in 1 percent ionagar or on filter paper. Homogenates were prepared from 50 g (wet weight) of the leafy portion of lettuce in 200 ml of double-distilled water by blending at low speed in a Waring blender in 3- to 5-second bursts. The homogenate was filtered through cheese cloth, precipitated with 5 percent (weight to volume) trichloroacetic acid (TCA), stirred 1 hour at 0°C. After centrifugation at 500*g* for 20 minutes at 5°C, the supernatant was extracted five or six times with diethyl ether. The aqueous layer was saved for recovery of sugars, amino acids, and organic acids. The ether layer was removed and evaporated under N_2, and residual TCA was removed by chloroform partitioned against double-distilled water and subsequent evaporation of the chloroform. The resulting oil was tested as the lipid fraction. Total lipids were extracted also by the method of Folch *et al.* (*13*). Sugars, amino acids, and organic acids were recovered from the aqueous layer by chromatography with Dowex-50 followed by *O*-(diethylaminoethyl) (DEAE)-cellulose. The loaded Dowex was washed with 0.01*N* HCl to elute organic acids and sugars subsequently separated on DEAE. Amino acids were eluted from the Dowex with 4*N* NH_4OH. Sugars were recovered from the DEAE void volume by washing with double-distilled water (*p*H 6.4), and organic acids were eluted with 4*N* formic acid. These fractions were reduced in volume by flash evaporation, and were then lyophilized and reconstituted in spring water (Arrowhead Puritas). The purity of each fraction was ascertained by thin-layer chromatography (*14*). Percentage recoveries were measured by using ^{14}C-labeled glycine, lactate, and glucose as internal standards. The recoveries were 62, 45, and 57 percent, respectively, for amino acids, organic acids, and sugars. The constituents of the amino acid fraction are shown in Table 2. The organic acid fraction contained malate and citrate, the sugar fraction contained fructose and glucose. No attempt was made to identify amino sugars or vitamins. Any cross-contamination of fractions or presence of minor components was below resolution by thin-layer chromatography. The ionagar blocks measured 4 by 1.5 by 1.5 cm; the amino acid fraction was reconstituted to an equivalence of 10 m*M* glutamate, the organic acid fraction to 9 m*M* malate plus citrate, and the sugar fraction to 10 m*M* glucose plus fructose. Lipids were dissolved in absolute ethyl alcohol and portions of these were allowed to evaporate on filter paper (Whatman No. 4).

Experimental groups		No. of replicates*	Responses	
Attractant	Control		Attractant	Control
None	None	13	20.9 ± 2.3 (14.3)†	20.5 ± 2.5 (14.2)
Ionagar	Ionagar	25	25.2 ± 1.1 (18.7)	27.5 ± 0.9 (21.7)
Homogenate	Ionagar	2	36.3 ± 1.2 (35.0)‡	7.1 ± 7.1 (3.0)
Amino acids	Ionagar	4	34.5 ± 2.1 (32.3)‡	24.9 ± 1.7 (18.0)
Organic acids	Ionagar	5	30.9 ± 3.3 (27.0)	25.9 ± 0.6 (19.2)
Sugars	Ionagar	5	24.1 ± 1.9 (20.2)	26.5 ± 2.2 (17.0)
Lipids	Filter paper	4	19.1 ± 4.5 (12.0)	24.2 ± 2.0 (17.0)
Folch lipids	Filter paper	5	27.9 ± 1.6 (22.2)	25.1 ± 1.7 (18.2)

*Ten snails per replicate. †Mean ± standard error of transformed values (actual percentages). ‡Statistical significance $P < .05$.

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