ter extracts of strains that did and did not react with the PGP antiserum were hydrolyzed for chemical analysis in 1*N* HCl for 8 hours; hydrolysis was carried out in evacuated tubes at 100°C. The samples were dried to remove HCl and treated with alkaline phosphatase prior to reduction and acetylation for analysis of the polyol and sugar alditol acetates by gas-liquid chromatography (*4*). A comparative chemical analysis of strains that contain LTA and those that do not is shown in Table 2; strains 1D (received as *S. mitis*) and 3G in which glycerol teichoic acid was detected immunochemically contained 20 and 25 percent glycerol, respectively; strains 72x41 and 72x35, in which no teichoic acid was detected immunochemically, contained 5 and 6 percent glycerol. In general, the glycerol:phosphate ratio hovered around 1:1, but the presence of ribitol in all extracts indicated that these extracts still contained nucleic acid derivatives; this was expected since the extraction procedure and enzyme treatment is only a first step in the purification. It is clear, however, that teichoic acid–positive strains have significantly higher quantities of glycerol.

The failure to detect glycerol teichoic acid among these strains confirms the observation of McCarty (*12*). In his original paper concerning the occurrence of the polymer in bacteria, he observed that more than 50 percent of *S. sanguis* and *S. viridans* (an older synonym sometimes used for *S. mitis* strains and other α-hemolytic streptococci) did not contain the antigen. However, these observations apparently have been forgotten in the many recent publications concerning the location and function of these interesting polymers. In *M. lysodeikticus*, which also does not have a teichoic acid, a lipomannan has been found that is speculated to serve the same function as the lipoteichoic acid (*13*). It has been suggested that such amphipathic molecules may be essential for growth of Gram-positive bacteria (*1*). The presence of small amounts of glycerol and phosphate in *S. mitis* suggests a lipid may be present that could be part of an amphipathic molecule, and studies aimed at isolating such a polymer are under way. However, the results of my studies indicate clearly that LTA is not present in *S. mitis*, and thus the polymer is not universally found in all Gram-positive bacteria. These observations suggest that this species has adapted to existence without LTA and, therefore, teichoic acids may not be as essential for all Gram-positive bacteria as had been speculated (*1*). On the other hand, the most notable feature of *S. mitis* is the apparent lack of physiological properties often associated with most other streptococci. For example, most *S. mitis* strains ferment only a few sugars and their cell walls contain little, if any, rhamnose; thus, as suggested earlier in this report, identification is often by exclusion. Could it be that the absence of teichoic acids, although not preventing growth, does act to limit the physiological activity of this species? Such limitation may occur through effects on permeability; this in turn may be related to autolysin activity which may be regulated by LTA (*2*). Further study of these ubiquitous oral bacteria may eventually provide the clues for a better understanding of the role of LTA in Gram-positive bacteria.

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Ultraviolet Radiation–Induced Chromosomal Abnormalities in Fetal Fibroblasts from New Zealand Black Mice

Abstract. *Fibroblasts from New Zealand Black mouse fetuses manifest increased frequency of chromosomal breaks and interchanges after exposure to ultraviolet radiation when compared with cells from BALB/c fetuses. This chromosomal instability is similar to what has been reported in cells from patients with xeroderma pigmentosum and may be related to the chromosomally abnormal clones and malignancy previously reported in adult New Zealand Black mice.*

Several inherited diseases in man are characterized by chromosomal fragility usually manifest as breaks, fragments, and interchanges and by increased frequency of malignancy (*1*). These chromosomal instability syndromes include as prototypes, Bloom's syndrome, Fanconi's anemia, ataxia telangiectasia, and xeroderma pigmentosum. In the first three diseases, chromosomal aberrations occur spontaneously. In contrast, in xeroderma pigmentosum, which is characterized by defective repair of ultraviolet radiation–induced alterations in DNA (*2*), the cytogenetic abnormalities are found only after exposure to ultraviolet radiation (*3*).

Understanding of the relation between chromosome abnormalities and malignancy in these syndromes could be advanced by the discovery of suitable ani-

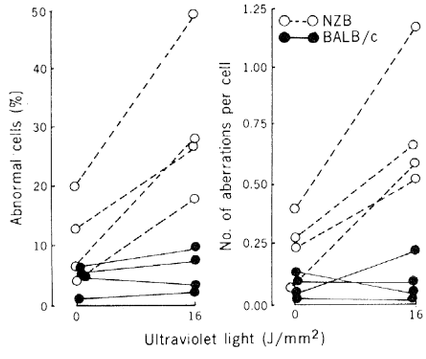

Fig. 1. Chromosomal abnormalities in cultured NZB and BALB/c fetal fibroblasts before and after exposure to ultraviolet radiation.

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Table 1. The effect of different doses of ultraviolet radiation on NZB and BALB/c fetal fibroblasts in vitro. The significance of increased frequency of abnormal cells after expoure to ultraviolet radiation compared to the frequency before exposure is indicated below the table.

Radiation dose (J/mm²)	Number of cells examined	Abnormal cells (%)	Abnormalities per cell					
			Breaks		Interchanges		Others	Total
			Chromatid	Chromosome	Chromatid	Chromosome		
			BALB/c cells					
	100	8 (8)	0.08	0.01				0.09
8	100	9 (9)	0.05	0.06	0.01			0.11
16	100	8 (8)	0.04	0.08	0.01	0.01		0.14
32	90	24 (26)*	0.31	0.18	0.20	0.01		0.70*
			NZB cells					
	93	7 (7.5)	0.04	0.06				0.10
8	100	17 (17)†	0.04	0.13	0.04	0.01	0.01	0.23†
16	100	18 (18)†	0.13	0.24	0.01	0.07		0.45*
32	100	57 (57)*	0.52	0.50	0.28	0.09		1.39*

*$P < .01$. †$P < .05$.

mal models. New Zealand Black (NZB) mice are characterized by high frequency of autoimmunity (*4*), spontaneous lymphoreticular malignancy (*5*), and increased susceptibility to the chemical carcinogen, 2-aminofluorene (*6*). In addition, spontaneously occurring chromosomal breakage in marrow cells has been described in one NZB colony (*7*). In contrast, other colonies lack these aberrations, but have mosaicism for spleen cell aneuploidy and euploidy (*8*). In all of these studies, the chromosomal aberrations were found only in older animals. We studied fetuses from one of the NZB colonies which apparently lacks spontaneously occurring chromosome fragility in spleen and marrow cells, and describe here the increased sensitivity of cultured fetal fibroblasts to ultraviolet radiation.

We used mice from the NZB/Sea colony as our experimental animals and BALB/c animals as controls (*9*). Metaphase spreads from cultured experimental and control fetal fibroblasts were analyzed without knowledge of their origin before and after exposure to ultraviolet radiation (*9*). Breaks and rearrangements of chromosomes, but not gaps, were scored as abnormal.

Chromosomal abnormalities were found more frequently in untreated NZB cells than in BALB/c cells, but the difference was not significant at the .05 level (Wilcoxin two-sample rank test) (Fig. 1). After exposure to a 16 J/mm² dose of ultraviolet radiation, BALB/c fibroblasts did not show an increase in the incidence of aberrations. In contrast, the incidence of chromosome abnormalities increased significantly in NZB fibroblasts exposed to radiation in each of four experiments ($P < .01$) (Fig. 1). The abnormalities detected included chromatid and chromosome breaks and interchanges (Fig. 2). There were 13 interchanges in 12,080 NZB chromosomes prior to ultraviolet radiation compared to 35 interchanges in 11,480 chromosomes after exposure to ultraviolet radiation ($P < .001$) (pooled results of four experiments; data not shown).

There was a direct relation between the frequency of abnormal cells and the dose of radiation (Table 1). At lower doses, increased frequency of chromosomal abnormalities was seen only in NZB cells. At the highest dose (32 J/mm²), both NZB and BALB/c cells exhibited a high incidence of abnormalities, but significantly more abnormal cells were found in NZB than in BALB/c fetal fibroblasts ($P < .01$).

The incidence of abnormal cells induced by 16 J of ultraviolet radiation per square millimeter was examined at 24, 48, and 72 hours after exposure. The frequency of abnormal cells was maximum at 24 hours after radiation, and then de-

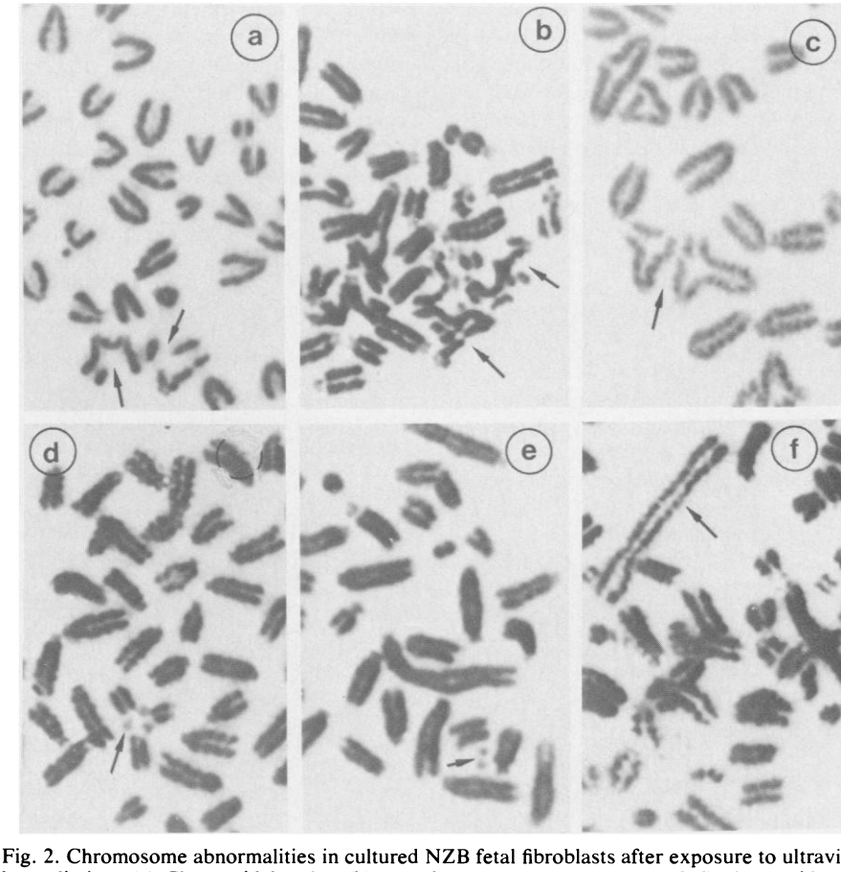

Fig. 2. Chromosome abnormalities in cultured NZB fetal fibroblasts after exposure to ultraviolet radiation. (a) Chromatid breaks; (b) complex rearrangements; (c and d) chromatid and chromosome exchange; (e) paired minute fragments; (f) rearranged abnormal chromosome.

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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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.
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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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