circulate in the peripheral blood (*12*). It is conceivable, therefore, that the knobs of *P. falciparum* contain several components, only one of which is required for attachment.

The knobs of *P. falciparum* were shown previously to be antigenic (*13*). Using the assay system described here we have recently identified a serum from an immune *Aotus* monkey (*14*) that abolishes attachment. It should therefore be possible to identify the adhesive component, or components, on the infected erythrocyte membrane and to investigate its immunogenic potential.

IROKA J. UDEINYA*
Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205
JOHN A. SCHMIDT
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases
MASAMICHI AIKAWA
Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 64108
LOUIS H. MILLER
Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases
IRA GREEN
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases

References and Notes

1. A. Bignami and G. Bastianelli, *Riforma Medica* **6**, 1334 (1890).
2. H. C. Clark and W. J. Tomlinson, in *Pathologic Anatomy of Malaria*. M. F. Boyd, Ed. (Saunders, Philadelphia, 1949), p. 874.
3. L. W. Scheibel, S. H. Ashton, W. Trager, *Exp. Parasitol.* **47**, 410 (1979).
4. L. H. Miller, *Am. J. Trop. Med. Hyg.* **18**, 860 (1969).
5. W. Trager, M. A. Rudzinska, P. C. Bradbury, *Bull. WHO* **35**, 883 (1966); S. A. Luse and L. H. Miller, *Am. J. Trop Med. Hyg.* **20**, 655 (1971).
6. E. A. Jaffe, R. L. Nachman, C. G. Becker, C. R. Minick, *J. Clin. Invest.* **52**, 2745 (1973).
7. C. L. Diggs, K. Joseph, B. Flemmings, R. Snodgrass, F. Hines, *Am. J. Trop. Med. Hyg.* **24**, 760 (1975).
8. W. Trager and J. B. Jensen, *Science* **193**, 673 (1976).
9. A Brazilian strain of *P. falciparum* was kindly supplied by M. McNeil, Walter Reed Army Institute of Research, and a Thai strain, T_2, by R. Nussenzweig, New York University School of Medicine.
10. M. A. Rudzinska and W. Trager, *J. Protozool.* **15**, 73 (1968); R. S. Desowitz, L. H. Miller, R. D. Buchanan, B. Permpanich, *Trans. R. Soc. Trop. Med. Hyg.* **63**, 198 (1969); H. N. Fremount and L. H. Miller, *Am. J. Trop. Med. Hyg.* **24**, 1 (1975).
12. D. H. Smith and R. D. C. Theakson, *Ann. Trop. Med. Parasitol.* **64**, 329 (1970).
13. A. Kilejian, A. Abati, W. Trager, *Exp. Parasitol.* **42**, 157 (1977); S. G. Langreth and R. T. Reese, *J. Exp. Med.* **150**, 1241 (1979).
14. Serum samples from immune *Aotus* were kindly supplied by J. D. Haynes, Walter Reed Army Institute of Research.

* Send reprint requests to Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Building 5, Room 112, Bethesda, Md. 20205.

5 January 1981; revised 27 March 1981

Female Feathering in Sebright Cocks Is Due to Conversion of Testosterone to Estradiol in Skin

Abstract. *Sebright cocks develop a female feathering pattern but revert to normal male feathering after castration. Administration of testosterone to castrated cocks causes male comb development and reappearance of female feathering. Dihydrotestosterone treatment supports development of a male comb but does not induce female feathering. Since testosterone but not dihydrotestosterone is converted to estradiol in the skin of the Sebright, the female feathering appears to be the result of increased conversion of testosterone to estradiol.*

In most chickens a profound difference in plumage develops between males and females at the time of sexual maturation. In the male the feathers of the neck, cape, back, and saddle are deeply fringed because of an absence of barbules on the ends of the feather barbs, and the feathers of the tail and neck hackle are long and curved. In the female most feathers have a solid vane with less fringing, and the feathers of the tail are short and stand erect. Development of the female feathering pattern is the result of a positive effect of estrogen, whereas formation of male plumage is independent of the action of gonadal hormones (male plumage develops in males and females after castration) (*1*).

In breeds carrying the henny-feathering trait, such as the Sebright bantam and Campine, plumage is identical in the two sexes and resembles that of the females of other breeds (*2*). Castration of such chickens causes the female feathering to revert to normal male plumage (*3*), and treatment of castrated males with testosterone causes a return of the female feathering (*4*). Transplantation of the testis from the Sebright cock to the castrated Leghorn chicken results in development of a male comb but does not alter normal male feathering in the Leghorn, implying that the testis of the Sebright produces normal male hormones (*5*). Danforth and colleagues (*6*) showed that when skin is transplanted from Sebright or Campine cocks to normal males, female feathering persists in the transplanted skin whereas feathering is always of the donor type in transplants of skin from normal males to males with the henny-feathering trait. Therefore, the defect must reside in the skin itself and is apparently due to the fact that testosterone acts aberrantly as an estrogen in the skin of birds with this trait. This could occur by either of two mechanisms. Androgen could act directly as an estrogen or could be converted to estrogen in increased amounts.

The conversion of androgen to estrogen in peripheral tissues such as skin is a significant source of estrogen formation in humans (*7*), and we recently reported that estrogen formation is markedly increased in slices of skin and skin appendages (*8*) and in fibroblasts cultured from the skin of Sebright and Campine birds (*9*). Consequently, we proposed that this increased estrogen synthesis causes female feathering in males with the trait. If this thesis is correct, injecting the castrated Sebright cock with androgens that can be converted to estrogens (such as testosterone) should induce female feathering, whereas androgens that cannot be converted to estrogens (androgens with 5α-reduced A rings, such as dihydrotestosterone) should virilize the male secondary sex characteristics in-

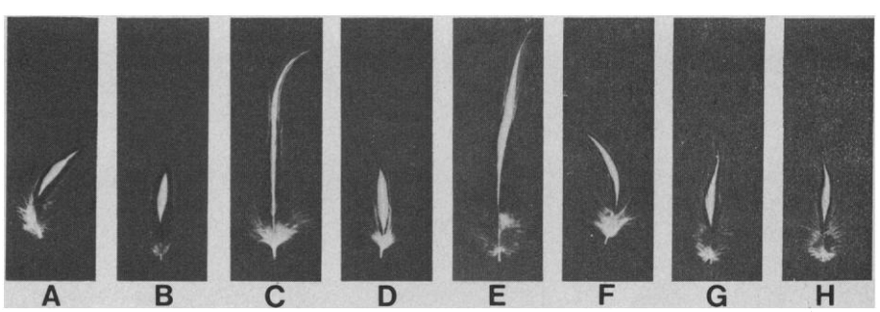

Fig. 1. Individual neck hackle feathers from Sebright bantam chickens subjected to various hormonal regimens for 3 months. (A) Intact male; (B) intact female; (C) castrated male treated with 0.1 ml of triolein per day; (D) castrated male treated with 100 μg of estradiol per day; (E) castrated male treated with 1 mg of dihydrotestosterone per day; (F) castrated male treated with 1 mg of testosterone per day; (G) castrated male treated with dihydrotestosterone (1 mg/day) plus estradiol (100 μg/day); (H) castrated male treated with testosterone (1 mg/day) plus estradiol (100 μg/day). Each dose was injected in 0.1 ml of triolein into the breast muscle.

0036-8075/81/0731-0557$00.50/0

cluding comb and wattle but should not alter the male feathering pattern.

Previous studies addressing this issue have yielded conflicting results. Callow and Parkes (*10*) and Deanesly and Parkes (*11*) reported that the administration of androsterone or 5α-androstane-3α, 17β-diol [weak, 5α-reduced androgens that can be converted to dihydrotestosterone in vivo (*12*)] resulted in maintenance of male feathering whereas testosterone induced female feathering—a finding in keeping with the concept that testosterone acts to feminize by serving as a precursor for estrogen synthesis. However, Koch (*13*), unable to confirm these findings, reported that androsterone treatment resulted in female feathering in the castrated Sebright cock, suggesting that androgens have direct estrogenic actions in this breed.

Table 1. Summary of the effects of estradiol, dihydrotestosterone, and testosterone on phenotypic development of comb and feathers in the Sebright bantam chicken.

Sex	Castration	Hormone therapy	Phenotype	
			Comb	Feathers
Male	No	None	Male	Female
Female	No	None	Female	Female
Male	Yes	None	Female	Male
Male	Yes	Estradiol	Female	Female
Male	Yes	Dihydrotestosterone	Male	Male
Male	Yes	Testosterone	Male	Female
Male	Yes	Dihydrotestosterone plus estradiol	Male	Female
Male	Yes	Testosterone plus estradiol	Male	Female

We therefore compared the effects of 3 months of treatment with testosterone or dihydrotestosterone on feathering and comb development in silver Sebright cocks (Halbach Poultry Farm, Waterford, Wisconsin) castrated at 2 weeks of age. Dihydrotestosterone and testosterone were chosen for this comparison for two reasons. Testosterone is the principal androgen secreted by the testis; it can act directly as an androgen or be reduced to dihydrotestosterone, a potent intracellular androgen in many tissues. Alternatively, testosterone can be converted to estrogen in peripheral tissues (*14*). Thus the effects of testosterone are both androgenic and estrogenic actions. In contrast, dihydrotestosterone cannot be converted to estrogen (*15*) and acts in the intact animal only as an androgen (*14*).

The feathers of the neck hackle are similar in size and configuration in the intact male (Fig. 1A) and female (Fig. 1B) and in the castrated male treated with either estradiol (Fig. 1D) or testosterone (Fig. 1F). In contrast, the neck feathers in the castrated male (Fig. 1C) and the castrated male treated with dihydrotestosterone (Fig. 1E) are long and more widely fringed, like those in normal male chickens (*1*).

The differences in the effects of testosterone and dihydrotestosterone on tail feather development are equally striking. In the intact Sebright male (Fig. 2A) and female (Fig. 2B) and in the castrated male treated with estradiol (Fig. 2D) or testosterone (Fig. 2F), the tail feathers are short and stand upright. In the castrated male (Fig. 2C) and in the castrated male treated with dihydrotestosterone (Fig. 2E), some tail feathers are long and curved, giving the tail a male configuration. In contrast to the divergent effects of testosterone and dihydrotestosterone on feathering, both androgens promoted development of the comb and wattle (Fig. 2, E and F). Comb development in the castrated control (Fig 2C) was similar to that in the intact female (Fig. 2B) and in the castrated male given estradiol (Fig. 2D). These findings are in keeping with the view that the henny-feathering trait is caused by the conversion of tes-

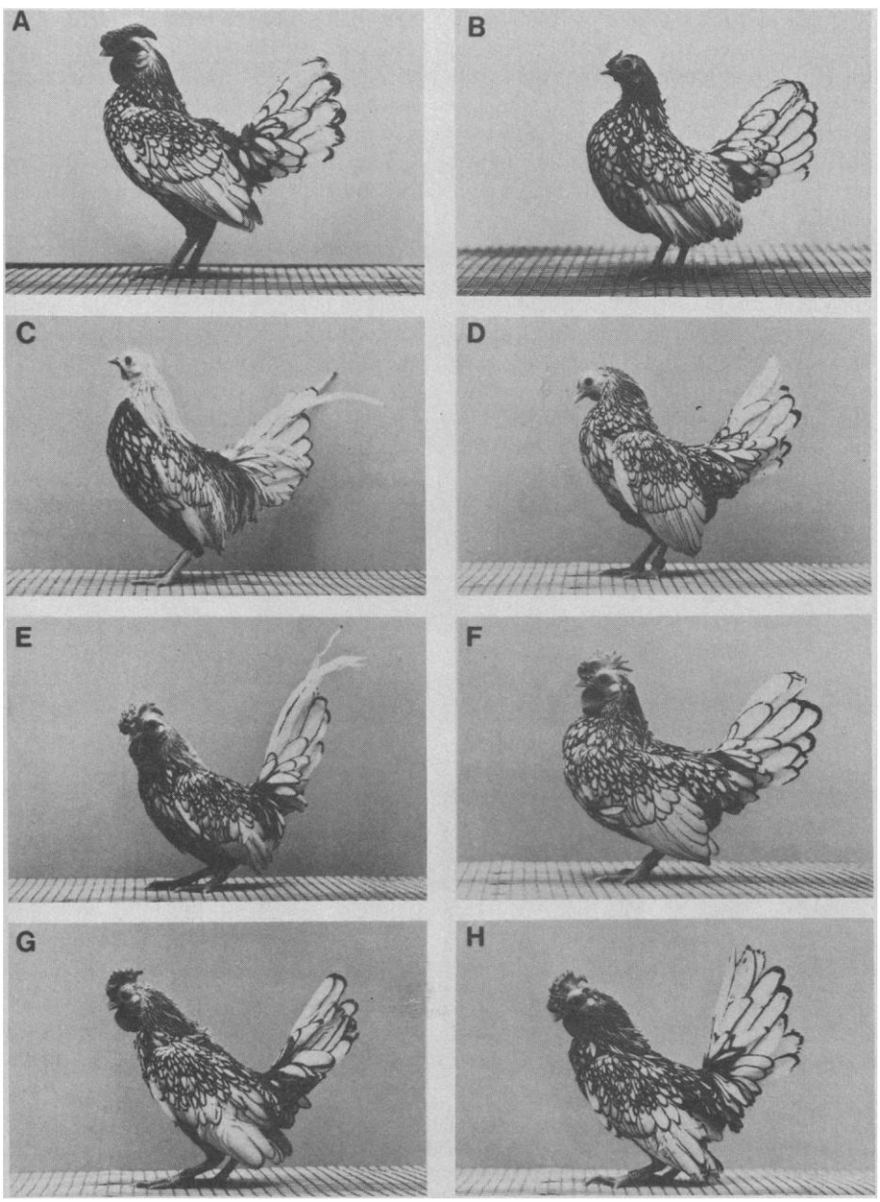

Fig. 2. Intact male and female and castrated male Sebright bantam chickens subjected to various hormonal regimens for 3 months. Identifications are given in the legend to Fig. 1.

tosterone to estrogen in the skin of affected birds.

The effects of combinations of androgen and estrogen on feathering were also compared. Henny feathering was as striking in castrated birds treated with either estradiol plus dihydrotestosterone or testosterone plus estradiol (Figs. 1, G and H, and 2, G and H) as in birds treated with estradiol or testosterone alone. The effects of the hormonal regimens on feather and comb development are summarized in Table 1.

Since dihydrotestosterone, which cannot be converted to estrogen, supports the development of a male comb but does not cause female feathering in the castrated Sebright male, and since treatment with dihydrotestosterone does not interfere with estrogen-induced feminization of feathers, we conclude that increased conversion of testosterone to estrogen in skin (*8, 9*) is the cause of female feathering in Sebright and Campine cocks. These results not only explain the phenomenon of female feathering in these chickens but also resolve the uncertainty about the endocrinology of the Sebright male in that they confirm prior reports that 5α-reduced androgens allow male feathering in the castrated male Sebright (*10, 11*). The failure of Koch (*13*) to demonstrate male feathering after administration of 5α-reduced androgens to castrated Sebright cocks might be due to regeneration of testicular tissue from retained remnants, which is common in male birds after castration (*16*).

Analysis of other mutations that alter the metabolism of steroid hormones in peripheral tissues, such as steroid 5α-reductase deficiency, has provided valuable insight into the molecular mechanisms by which the hormones normally act within cells and into the pathophysiology that results from the aberrant metabolism (*17*). Elucidation of the molecular mechanisms responsible for increased estrogen synthesis in birds with the henny-feathering trait may provide insight into the regulation of estrogen formation in peripheral tissues in normal individuals and in humans with increased estrogen synthesis in peripheral tissues (*18*).

The development of the henny-feathering trait in these chickens is different than that in some other birds. In the pigeon, guinea fowl, orange weaver, song sparrow, and snipe, for example, feathering in the male and female is similar and resembles that of females of other species, but in these birds henny feathering persists in both sexes following bilateral castration (*1*). In such species, female feathering in males must be due to some mechanism other than the conversion of testicular androgens to estrogens in skin.

FREDRICK W. GEORGE
JANET F. NOBLE
JEAN D. WILSON

Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas 75235

References and Notes

1. L. V. Domm, in *Sex and Internal Secretions*, E. Allen, C. H. Danforth, E. A. Doisy, Eds. (Williams & Wilkins, Baltimore, 1939), pp. 227–327.
2. W. B. Tegetmeir, *The Poultry Book* (Routledge & Kegan Paul, London, 1867), pp. 241–247.
3. T. H. Morgan, *Proc. Soc. Exp. Biol. Med.* **15**, 3 (1917); *Endocrinology* **4**, 381 (1920).
4. T. F. Gallagher, L. V. Domm, F. C. Koch, *J. Biol. Chem.* **100**, 47 (Abstr.) (1933).
5. H. A. Roxas, *J. Exp. Zool.* **46**, 63 (1926).
6. C. H. Danforth, *Proc. Soc. Exp. Biol. Med.* **26**, 86 (1928); *Biol. Gen.* **6**, 99 (1930); in *Sex and Internal Secretions*, E. Allen, C. H. Danforth, E. A. Doisy, Eds. (Williams & Wilkins, Baltimore, 1939), pp. 328–350; *Proc. Soc. Exp. Biol. Med.* **32**, 1474 (1935); *Essays in Biology* (Univ. of California Press, Berkeley, 1943), pp. 159–167; *Yale J. Biol. Med.* **17**, 13 (1944).
7. P. K. Siiteri and P. C. MacDonald, *Handb. Physiol.* **2**, 615 (1973).
8. F. W. George and J. D. Wilson, *J. Clin. Invest.* **66**, 57 (1980).
9. M. Leshin, F. W. George, J. D. Wilson, *J. Biol. Chem.* **256**, 4341 (1981).
10. R. K. Callow and A. S. Parkes, *J. Exp. Biol.* **13**, 7 (1936).
11. R. Deanesly and A. S. Parkes, *Q. J. Exp. Physiol.* **26**, 393 (1937).
12. N. Bruchovsky, *Endocrinology* **89**, 1212 (1971).
13. F. C. Koch, *Physiol. Rev.* **17**, 153 (1937).
14. J. D. Wilson, *Handb. Physiol.* **5**, 491 (1975).
15. E. A. Thompson, S. B. Bolton, P. K. Siiteri, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 1160 (Abstr.) (1971); C. Faiman and J. S. D. Winter, *J. Clin. Endocrinol. Metab.* **39**, 631 (1974); G. Schaison, M. Renoir, M. Lagoguey, I. Mowszowicz, *ibid.* **51**, 1133 (1980).
16. L. H. Schwarte, in *Diseases of Poultry*, H. E. Biester and L. H. Schwarte, Eds. (Iowa State College Press, Ames, ed. 2, 1948), pp. 961–974.
17. J. E. Griffin and J. D. Wilson, *N. Engl. J. Med.* **302**, 198 (1980).
18. D. L. Hemsell, C. D. Edman, J. F. Marks, P. K. Siiteri, P. C. MacDonald, *J. Clin. Invest.* **60**, 455 (1977).
19. We thank B. Newton for castrating the Sebright cocks. Supported by NIH grant AM03892.

23 March 1981; revised 18 May 1981

Copper Deficiency Suppresses the Immune Response of Mice

Abstract. *Mice fed a purified diet low in copper display anemia, hypoceruloplasminemia, depressed concentrations of liver copper, and elevated concentrations of liver iron. An impaired humoral-mediated immune response (decreased numbers of antibody-producing cells) is observed in mice with severe as well as marginal copper deficiency. The magnitude of this impairment is highly correlated with the degree of functional copper deficiency (hypoceruloplasminemia).*

Nutrition plays an essential role in immunologic function. Malnutrition due to deficiencies of protein, calories, vitamins, or trace elements leads to impairment of both humoral immunity (antibody production) and cell-mediated immunity (*1*).

In 1968 Newberne *et al.* (*2*) reported increased mortality in copper-deficient rats exposed to *Salmonella typhimurium*. Recurrent pulmonary and urinary tract infections are common in most infants with Menkes syndrome, a genetic disorder resulting in copper deficiency (*3*). In this lethal syndrome, death is most often caused by bronchopneumonia. Copper deficiency in humans, although rare, is accompanied by bacterial infections (*Escherichia coli*, *Staphylococcus aureus*), diarrhea, and bronchopneumonia (*4*). Since copper is required for a variety of metabolic functions, its deficiency leads to many pathophysiological expressions besides infection.

We investigated the possibility that copper, like certain other trace elements, is necessary for immunocompetence. Since dietary copper deficiency had not been studied in mice, our initial experiments were designed to establish an appropriate model. Our plan was to produce animals with less than normal copper stores by feeding C58 mice a purified diet low in copper and then to expose them to a foreign antigen (sheep erythrocytes) to evaluate their antibody-producing capabilities by the Jerne-Nordin plaque technique (*5*). Several preliminary studies were conducted (*6*), after which we selected a method that allowed the greatest survival of animals and produced animals exhibiting a full range of deficiency signs. All mice were fed a purified diet low in copper from the time of parturition. Control animals were supplemented with $CuSO_4$ in their drinking water. The latter were indistinguishable in terms of a variety of biochemical and immunologic parameters, from mice raised by dams fed a nonpurified diet containing adequate copper. The response to our dietary treatment was quite variable. Therefore, for comparative purposes, the copper-deficient mice were divided into two groups based on residual levels of the copper-dependent enzyme ceruloplasmin: mice with activities below 3 U/liter ($-Cu_1$) and mice with activities above 3 U/liter ($-Cu_2$). The variability may have been due to the fact that our purified diet was not entirely devoid of copper and thus was probably sufficient for some mice in a phase of slow growth.

0036-8075/81/0731-0559$00.50/0