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.

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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.

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Table 1. Effects of dietary copper deficiency in C58 mice. On the day of parturition, C58 mouse dams were switched from a nonpurified diet (Mouse Chow, Ralston Purina) containing 12 mg of copper per kilogram to a purified mouse diet formulated to omit copper from the salt mix (modified AIN-76, ICN Nutritional Biochemicals) and containing 0.5 mg of copper per kilogram. Approximately half the dams were given supplemental copper (20 μg/ml) in their drinking water, while the remaining dams were given deionized water to drink. The pups were weaned at 3 weeks of age and transferred to stainless steel cages. For 4 to 6 weeks they were maintained on the diets that had been given to their respective dams. Then the mice were injected with sheep red blood cells and decapitated 4 days later to evaluate humoral-mediated immunity (*5*); blood was collected in acid-washed tubes. A small portion was removed to measure hemoglobin as cyanmethemoglobin with Drabkin reagent (*13*); the remainder was allowed to clot and the serum was assayed in duplicate for ceruloplasmin activity, with *o*-dianisidine used as the substrate (*14*). The spleen was removed to determine the number of antibody-producing cells, and the liver was wet-ashed in HNO_3 to determine total copper and iron by atomic absorption spectroscopy. Values are means ± standard deviations for the indicated number of supplemented (+Cu) and unsupplemented (−Cu) mice from seven individual experiments. Values in the same row for a given sex, not sharing a common superscript, are significantly different from one another at $P < .01$.

Parameter	Male			Female		
	+Cu (N = 12)	$-Cu_1$ (N = 6)	$-Cu_2$ (N = 5)	+Cu (N = 9)	$-Cu_1$ (N = 3)	$-Cu_2$ (N = 14)
Body weight (g)	23.2 ± 3.7[a]	15.6 ± 2.3[b]	17.8 ± 1.6[b]	17.6 ± 2.2[x]	13.1 ± 0.83[y]	15.2 ± 4.2[x]
Spleen weight (mg)	152 ± 44[a]	291 ± 132[b]	114 ± 17[a]	120 ± 32[x]	177 ± 63[x]	115 ± 40[x]
Hemoglobin (g/dl)	14.6 ± 0.4[a]	7.14 ± 2.83[b]	13.1 ± 1.2[c]	14.7 ± 0.79[x]	5.15 ± 1.68[y]	13.3 ± 0.49[z]
Ceruloplasmin (U/liter)	23.4 ± 2.6[a]	0.72 ± 0.99[b]	7.32 ± 5.60[c]	22.8 ± 1.9[x]	0.16 ± 0.27[y]	10.9 ± 3.54[z]
Liver Cu (μg/g)	5.86 ± 0.78[a]	2.05 ± 0.49[b]	3.13 ± 0.50[c]	3.89 ± 0.75[x]	1.58 ± 0.17[y]	3.32 ± 0.24[x]
Liver Fe (μg/g)	61.7 ± 13.1[a]	255 ± 122[b]	126 ± 49[b]	86.3 ± 20.1[x]	296 ± 51[y]	76.4 ± 14.2[x]
Antibody-producing cells per 10^6 splenocytes	1004 ± 132[a]	175 ± 96[b]	644 ± 165[c]	874 ± 254[x]	23.3 ± 5.8[y]	793 ± 315[x]

By early adulthood, more of the males than females expressed severe symptoms characteristic of copper deficiency (anemia, hypoceruloplasminemia, decreased liver copper, and increased liver iron). However, there were significantly fewer antibody-producing cells in copper-deficient mice of both sexes than in the copper-supplemented mice ($P < .01$, Student's t-test) (Table 1). In $-Cu_1$ mice there were, compared to the controls, significant decreases in body weight (decreases of 33 percent in males and 26 percent in females), hemoglobin (51 and 65 percent), ceruloplasmin (97 and 99 percent), liver copper (65 and 59 percent), and antibody-producing cells (83 and 97 percent), whereas there were significant increases in spleen weight (191 and 148 percent) and liver iron (413 and 343 percent) (Table 1). These data establish that humoral-mediated immunity is severely impaired in mice that are copper-deficient by the criteria used to define deficiency in other species.

Compared to the controls, $-Cu_2$ males also had significant decreases in body weight (23 percent), hemoglobin (10 percent), ceruloplasmin (69 percent), liver copper (47 percent), and number of antibody-producing cells (36 percent) (Table 1). Liver iron was elevated, but spleen weight was not. The values for the $-Cu_2$ females were not significantly different from control values except for a mild anemia, hypoceruloplasminemia, and modest (15 percent) liver copper depletion ($P < .05$). Antibody production seemed normal. In the $-Cu_2$ mice there was an apparent relation between the degree of copper deficiency and the impairment of immune function. [This correlation is not evident when the values are averaged (Table 1).] Serum ceruloplasmin activity in the copper-deficient mice was highly correlated with the residual capacity to produce antibodies against sheep red blood cells in both males (r = .861) and females (r = .919) (Fig. 1). Each regression line indicates complete loss of antibody production when serum ceruloplasmin activity is no longer detectable. The decrease in liver copper and hemoglobin and the increase in liver iron were also correlated with the degree of immune impairment. Thus, it appears that in addition to its other physiological roles, copper is necessary for proper immune function.

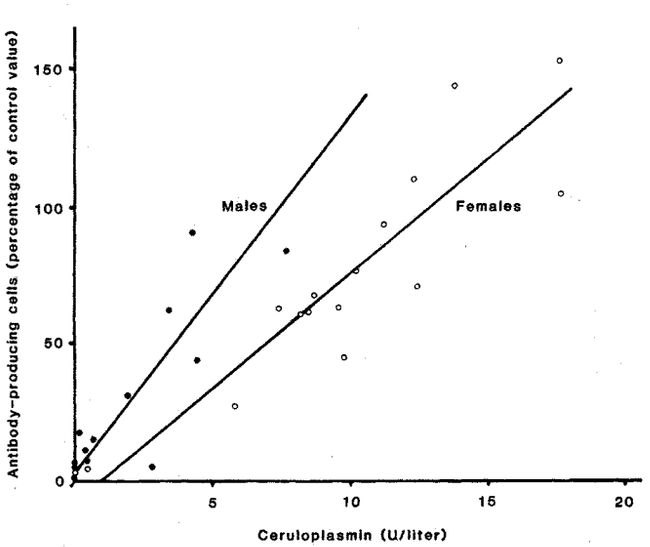

Fig. 1. Relation between antibody formation and copper deficiency in young adult C58 mice. Humoral-mediated immunity was evaluated by the Jerne-Nordin assay in splenocytes harvested 4 days after injection of sheep red blood cells, and the response of copper-deficient mice was compared to that of copper-supplemented mice. Serum ceruloplasmin activity was determined in the same mice to evaluate the degree of copper deficiency. A total of 13 copper-deficient males (●) and 17 females (○) from seven experiments were analyzed. Linear regression analysis was performed and a significant ($P < .01$) regression was observed for both males (r = .861) and females (r = .919) (*15*). The two regression lines determined by the method of Brace (*16*), have significantly different slopes ($P < .05$) (*15*). The average ceruloplasmin number of antibody-producing cells was 1004 per 10^6 splenocytes (100 percent) for control males and 874 for control females, and the activity was 23.4 U/liter for control males and 22.8 for control females (Table 1).

The production of antibodies against sheep red blood cell antigens by the plasma cells of the spleen (B cells) requires the cooperation of the thymus-dependent system (T cells). Pedroni *et al.* (*7*) reported impairment in T cell production in a patient with Menkes syndrome, but this was refuted by Sullivan and Ochs (*8*), again based on a single patient. Further immunological studies

are necessary to establish the cellular and molecular basis of the defect in antibody production in copper-deficient mice.

The effect of copper deficiency on the immune response appears to be distinct from the effects of deficiencies of other trace metals (zinc and iron) known to affect immune function. Zinc deficiency causes a decrease in the number of antibody-producing cells which appears to be dependent on T cell helper function (*9*). In zinc-deficient mice, the spleen and thymus are atrophic. Our copper-deficient mice, with impaired immune function, had normal or enlarged spleens (Table 1) and normal-appearing thymus glands. Furthermore, zinc metabolism is unaltered during copper deficiency (*10*). Iron deficiency appears to alter humoral-mediated immunity (*11*); thus the defects in iron metabolism in our copper-deficient mice may explain the impairment in antibody production. This is unlikely, however, since some $-Cu_2$ mice that had only mild anemia and normal liver iron levels still had impaired immune response (Table 1 and Fig. 1). Multiple deficiencies in copper, iron, and zinc may occur in humans and result in impaired immunity due to a combination of mechanisms.

The many physiological roles of copper are evidenced by the numerous functional and structural alterations caused by its deficiency. Extensive investigations into the biochemical mechanisms have led some to theorize that reductions in copper-dependent enzymes such as ceruloplasmin, cytochrome oxidase, superoxide dismutase, lysyl oxidase, tyrosinase, and dopamine β-hydroxylase are responsible for the pathological expressions of copper deficiency (*12*). We do not know which, if any, of these enzymes might be involved in the immune response. However, it is clear that copper is another nutritional factor necessary for a competent immune system.

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Role of Golgi Apparatus in Sorogenesis by the Cellular Slime Mold *Fonticula alba*

Abstract. *Aggregating cells of the cellular slime mold* Fonticula alba *form a volcano-shaped fruiting structure which at maturity bears its spores apically in a globose mucous mass. Numerous dictyosomes forming in the sorogenic cells are involved in the accumulation and deposition of stalk material.*

In *Fonticula alba*, a recently described cellular slime mold (*1*), the trophic cells bear certain resemblances to the amoebas of both acrasid and dictyostelid cellular slime molds, but the form and organization of the sorocarp is unique. Major differences between acrasids and dictyostelids have been described elsewhere (*2*). The amoebas of *F. alba*, like those of the dictyostelids, form filose pseudopodia when placed under moist conditions. However, each cell, unlike those of the dictyostelids, has a nucleus with an inconspicuous nucleolus and mitochondria with discoid cristae similar to those of certain acrasids. The fruiting body with its volcano-shaped stalk is unlike any found among other cellular slime molds, with the possible exception of the acrasid *Guttulinopsis nivea* (*3*).

The fruiting stage begins when trophic amoebas cease feeding and form dense aggregates within or slightly on top of masses of amoebas and bacteria present on the agar surface. Once the cells have aggregated, they envelop themselves in a large mass of mucus-like material (Fig. 1A). A group of amoebas near the top of the aggregate rises upward as the developing stalk stretches into a slender projection (Fig. 1B). The base of the sorocarp is very thick and contains a large number of cells (Fig. 1E). As the amoebas move upward they synthesize and eject stalk material. While the cells are embedded in a portion of this material, the majority of it accumulates in the walls of the sorocarp, much of it within the basal area. The movement of cells leading to formation of the stalk neck continues until the neck achieves a height roughly proportional to the diameter of the base and then ceases. At this point, many of the cells inside the stalk cease making stalk material, become round, and develop spore walls around themselves. A small opening appears at the top of the stalk and the spores flow out into a large mucous mass atop the stalk (Fig. 1, C and D).

In order to fully understand the process of sorogenesis in *F. alba*, it is necessary to determine how the stalk is formed. Representative examples of the stages of sorogenesis were selected for observation by electron microscopy. Small plugs of the agar on which individual sorocarps were developing were covered with molten 2 percent pure agar. When the agar had solidified, completely immobilizing the sorocarps, the plugs were fixed in 2 percent glutaraldehyde buffered in 0.1*M* sodium cacodylate for 30 minutes at room temperature and then for 90 minutes at 4°C. The specimens were rinsed twice in 0.1*M* sodium caco-

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