Wasting Disease Induced with Cortisol Acetate: Studies in Germ-Free Mice

Abstract. *A marked difference in mortality was observed between germ-free mice and conventionally reared mice, both given a single injection of cortisol acetate. The incidence of death in germ-free mice was significantly lower than in conventionally reared mice. Mortality in germ-free mice treated with cortisol acetate was increased by monocontamination with* Escherichia coli.

Wasting syndromes can be produced by thymectomizing newborn mice soon after birth (*1*) or by treating newborn mice with allogenic lymphoid cells (*2*), multiple doses of certain bacterins (*3*), cortisol acetate (*4*), or estradiol (*5*). A similar disease can be produced in adult F_1 hybrid mice by injection with parental lymphoid cells (*6*), and in lethally irradiated adult mice by injection with allogenic bone marrow (*7*). These syndromes are characterized by marked wasting, ruffled fur, diarrhea, and, in many cases, death. A striking pathological finding common to these diseases is a diminution of lymphoid cells and immunological competence.

Although the pathogenesis of the various wasting syndromes is not completely understood, recent studies on these diseases in germ-free mice have provided evidence that infectious agents in the environment may contribute to the pathological changes and death of wasted animals. Wilson, Sjodin, and Bealmear (*8*) and, independently, McIntire, Sell, and Miller (*9*) have shown that wasting and death do not occur after neonatal thymectomy in germ-free mice. Similarly, Ekstedt and Nishimura (*3*) observed that, whereas conventionally reared mice became runted when treated with multiple doses of heat-killed streptococci or staphylococci, germ-free mice were much more resistant to the runting syndrome. In view of the similar responses to the germ-free state in the wasting disease induced with bacterins and in the post-neonatal thymectomy syndrome, it was of interest to determine the effect of the germ-free state on the development of the wasting disease induced by cortisol acetate, described by Schlesinger and Mark (*4*).

We used randomly bred, germ-free Swiss mice and conventionally reared Swiss mice (*10*). Litters were sized to contain between seven and ten mice, and all mice in a given litter received the same treatment. An aqueous suspension of either 0.25 or 0.50 mg of cortisol acetate (*11*) in 0.05 ml was administered subcutaneously in the neck region of mice 36 hours old. Only mice dying later than day 3 after injection were considered as dying of wasting disease.

A single injection of 0.25 mg of cortisol acetate into mice 36 hours old resulted in moderate symptoms characteristic of the wasting syndromes in both conventionally reared and germ-free mice. The skin was thin and wrinkled and the growth of hair was impaired. When the coat did develop it was sparse and ruffled. Most of the mice recovered after a variable period of time and reproduced normally. The incidence of deaths with a dose of 0.25 mg was low in both the conventionally reared and the germ-free groups (Table 1). Although the weight gained by germ-free mice injected with cortisol acetate approached that of the control groups, the weight gained by conventionally reared mice injected with cortisol acetate was retarded during a 40-day observation period.

When 0.50 mg of cortisol acetate was given to germ-free and conventionally reared mice at 36 hours of age, both groups showed severe signs of wasting and a marked failure to gain weight. The skin was extremely loose, wrinkled, and thin, and the growth of hair was markedly impaired. The mice exhibited a hunched posture and a characteristic high-stepping gait. However, with this dose, a striking difference in mortality between the germ-free and the conventionally reared groups was observed (Table 1). These data show a cumulative mortality of 90.6 percent (58/64) in conventionally reared mice as contrasted with 15.1 percent (5/33) in germ-free mice at day 40 after injection. By this time, the germ-free survivors had developed a normal appearance and later reproduced.

Table 1. The effect of cortisol acetate injected into conventional (C), germ-free (GF), and monocontaminated (MC) mice. Mice were injected with cortisol acetate at 36 hours of age and monocontaminated at 4 days of age.

Cortisol acetate dose (mg)	Status of mice	Mice* (No.)	Mean survival time (days after injection) ± S.E.
0.25	C	4/25	9.0
.25	GF	3/24	15.0
.50	C	58/64	9.9 ± 0.2
.50	GF	5/33	11.0 ± 1.4
.50	MC	13/16	9.2 ± 1.1
None	C	2/37	15.5
None	GF	1/14	5.0
None	MC	1/16	9.0

* Numerators are the numbers of mice dying of wasting disease; denominators are the numbers of mice treated.

To determine the effect of monocontamination on germ-free mice treated with cortisol acetate, a strain of *Escherichia coli*, usually found in abundance in our conventionally reared mice, was isolated from mouse fecal material for use in monocontamination experiments. A single injection of 0.50 mg of cortisol acetate was administered to germ-free mice at 36 hours of age. The mice treated with cortisol acetate were placed in small, litter-size isolators (*12*) and monocontaminated at 4 days of age by adding 10 ml of a 24-hour broth culture of *E. coli* to a 75-ml supply of sterile water. This monocontamination procedure was repeated every third day. Both germ-free mice injected with cortisol acetate and monocontaminated mice showed a low mortality, whereas the mortality in monocontaminated mice treated with cortisol acetate was comparable to that observed in conventionally reared mice injected with cortisol acetate (Table 1).

In view of the manifold effects of adrenal cortical hormones on various tissues and organs of the body, it is difficult to assess the role of these hormones in wasting syndromes. Adrenalectomy has been shown both to ameliorate (*13*) and to enhance (*14*) the development of wasting syndromes. Perhaps of particular significance is the fact that large doses of adrenal cortical hormones have a profound lymphocytolytic effect (*15*). It is likely that as a result of the primary effects of cortisol acetate, particularly lymphoid depletion, secondary pathological events occur in conventionally reared mice that do not occur in germ-free mice. The monocontamination experiments reported here suggest that such secondary pathological events can be produced by the microorganisms which constitute the normal flora of conventionally reared animals. Moreover, the role of microbial products and viruses (*16*) in the development of

pathological changes in wasting disease must be considered.

Finally, it is suggested that the members of the normal flora, and their ecological relationships, may play an important role in the pathogenesis of other wasting syndromes.

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References and Notes

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Hybridization Experiments: Evidence of Dissociation Equilibrium in Hemerythrin

Abstract. *Partial succinylation of hemerythrin alters its electrophoretic mobility even though it remains an octameric macromolecule. Mixtures of this modified protein and unmodified hemerythrin generate species of intermediate electrophoretic mobility. Such behavior provides strong evidence that the octameric macromolecule is in mobile equilibrium with monomeric subunits.*

Hemerythrin, the iron-containing (in a form other than heme iron), oxygen-carrying protein of sipunculids, interacts with iron-coordinating ligands (*1*) as well as with sulfhydryl-blocking reagents (*2*). These interactions are easily detected since the iron-coordinating ligands alter the visible spectrum of the protein, and the sulfhydryl-blocking reagents cause dissociation of the protein into subunits.

Since the reaction of the –SH groups with organic mercurials or *N*-ethylmaleimide does not change the spectrum of hemerythrin, these groups cannot be in the immediate environment of the iron. Nevertheless, *N*-ethylmaleimide does not react with the –SH groups of hemerythrin in the absence of iron-coordinating ligands whereas the –SH groups become readily accessible in the presence of N_3^- ion (*1*). Thus, an interaction at one locus on the protein affects the reactivity of a second, and we have a clear example of a cooperative interaction.

A molecular interpretation of this cooperative interaction is provided by the assumption that native octameric hemerythrin is always in equilibrium with a very small amount of the monomeric form (Fig. 1). The key role of the –SH groups in preserving the native structure of hemerythrin suggests that these groups take part in some intra- or intermolecular interaction in the octamer. It seems reasonable to assume that these groups are more exposed in separated subunits. Indeed there is experimental evidence (*3*) for the masked character of –SH groups in native hemerythrin and for their availability in 8*M* urea, where dissociation occurs. Since ions such as N_3^- are bound more strongly by monomeric hemerythrin (produced by reaction with *N*-ethylmaleimide) than by octameric (*1*), these ions should shift the dissociation equilibrium (Fig. 1) to the right, increase the concentration of exposed –SH groups and hence accelerate the rate of reaction with sulfhydryl-blocking reagents.

This explanation is not the only one possible, however; a conformational rearrangement (without dissociation) of the octamer might be generated by the binding of ligands, and such a rearrangement could be responsible for the cooperative interaction.

We have searched, therefore, for experiments that might provide evidence in favor of one of these alternative explanations. In particular we have attempted to design an experiment that might detect very small amounts of subunit in equilibrium with octamer, if such an equilibrium does exist (*4*).

Such an experiment became feasible when we found that mildly succinylated hemerythrin remains in the octameric form. Succinylated, octameric hemerythrin was obtained by two methods. In the first, the –SH group of the protein was protected with a mercurial, the resulting monomer was succinylated, and the modified octamer was regenerated by removal of the mercurial. Modified octamer was also obtained by succinylating the native hemerythrin with a small amount of succinic anhydride in relation to the amount of protein. Either procedure

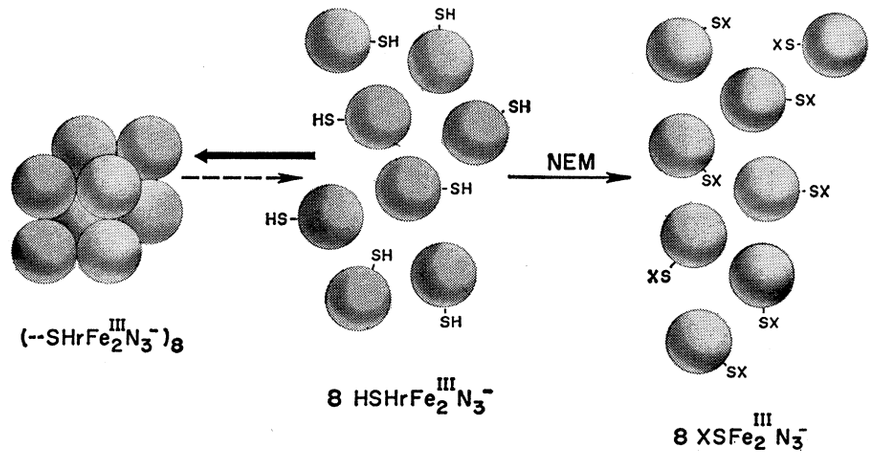

Fig. 1. Representation of equilibrium between intact hemerythrin (as azide complex) and its subunits, showing also the shift to monomers when *N*-ethylmaleimide combines with the sulfhydryl group.