embedded for optical and electron microscopic examination.

Clusters of lymphocytic cells could be found surrounding macrophages in both lymph nodes and spleen from all animals, but with a greater degree of frequency in the immunized animals. These clusters were scattered throughout the medullary areas of the lymph nodes and in the red pulp of the spleen, particularly in those areas of the red pulp adjacent to the mantle layer and the cords of the white pulp. It must be emphasized that the majority of the lymphocytic cells were not in such an arrangement. At an optical level, fusion of the cytoplasm of the macrophages with the surrounding lymphocytic cells could not be demonstrated. However, examination of these cells in the electron microscope revealed areas of direct communication between the cytoplasm of the macrophages and some of the immediately adjacent lymphocytic and plasma cells (Fig. 1). The cytoplasmic membranes of the two cells formed a continuous structure. Within this connecting corridor of cytoplasm small particles of the size of ribosomes could be resolved. There was no evidence of interchange of any other structures in these preliminary examinations. The transfer of antigenic molecules between macrophages and lymphocytes was not seen when horse ferritin was used as an electron-dense antigen. In fact, heavy-metal, labeled antigen was not identified in any of the known antibody-producing cells.

Direct communication between the macrophage and its satellite lymphocytic cells was not a common occurrence. Only one or two of the lymphocytic cells in the cluster showed this direct physical connection with the macrophage. In the immunized animals there were more cytoplasmic connections. The small number of cytoplasmic connections in any given "clone" may be the result of poor sampling. Extensive serial or closely adjacent sectioning has not been satisfactorily accomplished. However, these bridges may last for only a short time, as shown by Aronson (*7*) in the case of cytoplasmic bridges between phagocytic cells.

This evidence, added to that of others, suggests that there is a transfer of cytoplasmic content from the macrophage to lymphocytic cells, and that this exchange may be concerned with the transfer of ribosomal particles. It is reasonable to presume that the connections between these cells would allow for an easy transfer of such material.

At least two morphologically different cells can interact with the macrophage in lymphoid tissue. It is interesting to speculate on this point, since there is evidence that morphologically different types of cells produce 19*S* and 7*S* antibody, a large mononuclear cell having the features of a cell of the lymphocytic series and the plasma cell (*10*).

M. D. SCHOENBERG, V. R. MUMAW
R. D. MOORE, A. S. WEISBERGER
Institute of Pathology and *Department of Medicine, Western Reserve University, Cleveland 6, Ohio*

References and Notes

1. In this report the term *macrophage* applies to those cells of the reticuloendothelial system in the spleen and lymph nodes that are capable of phagocytosis. Some authors prefer the term *histiocyte*.
2. S. Amano, *Trans. Soc. Path. Japan* **35**, 42 (1946).
3. J. P. Thiery in *Symposium on Cellular Aspects of Immunity*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston, 1960), p. 59.
4. I. A. Sharp and R. G. Bursell, *Nature* **188**, 74 (1960).
5. M. Fishman, R. A. Hammerstrom, V. P. Bond, *ibid.* **198**, 549 (1963).
6. E. Undritz, *Folia Haematol.* **70**, 32 (1950).
7. M. Aronson, *J. Exptl. Med.* **118**, 1083 (1963).
8. M. Fishman, *ibid.* **114**, 837 (1961).
9. J. S. Garvey and D. H. Campbell, *ibid.* **105**, 361 (1957).
10. M. Schoenberg, J. Rupp, R. Moore, *Brit. J. Exptl. Pathol.*, in press.
11. Supported by grants RG 7200 and AM 07161, U.S. Public Health Service.

18 December 1963

Wasting Disease Induced in Young Mice by Administration of Cortisol Acetate

Abstract. *A single injection of cortisol acetate into young mice induced a wasting syndrome similar to that observed in runt disease and in the post-thymectomy syndrome. The course of the disease was less severe if the dose of the drug was decreased or if the animals were older at the time of injection.*

Wasting syndromes occur in newborn mice injected with allogeneic (*1*) lymphoid cells (runt disease) (*2*), in F_1 hybrids injected with parental lymphoid cells (*3*), in lethally irradiated mice injected with allogeneic bone marrow cells (secondary disease) (*4*), and in mice thymectomized shortly after birth (*5*). These syndromes are all characterized by progressive weight loss, ruffling of the fur, diarrhea, and death. A pathological finding that is common to all of these types of wasting is profound atrophy of the lymphoid organs. It has been suggested that this lymphoid depletion results in widespread metabolic dysfunction leading to the fatal wasting (*6*).

Although adrenal corticosteroids are known to cause lymphoid depletion (*7*), the role of these hormones has not been delineated with relation to the wasting diseases. In this study we investigated the effects of cortisol acetate (hydrocortisone acetate) on mice of various ages, the conditions under which a wasting disease can be produced, and the correlation between this and other types of wasting disease on the basis of morphologic criteria.

Mice of various strains, mostly C3H, were used throughout the experiment. Cortisol acetate in aqueous suspension was administered subcutaneously in the neck region. Animals were either allowed to pursue the natural course of the disease or were killed at varying time intervals after drug administration.

A single injection of 0.25 mg of cortisol acetate into 1-day-old mice resulted in a wasting syndrome strikingly similar to that developed in runt disease and in the post-thymectomy syn-

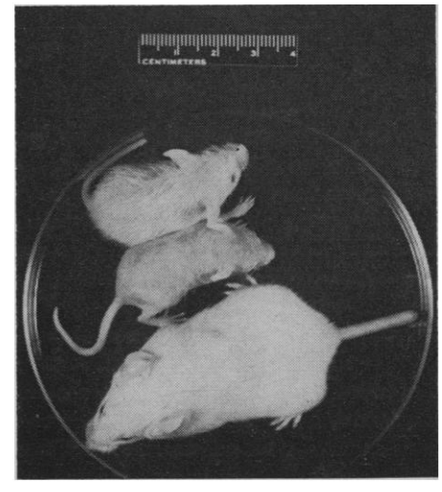

Fig. 1. Littermates (Swiss mice) age 27 days. The two mice with wasting disease were treated with 0.25 mg of cortisol acetate at 5 days of age; the third mouse served as an untreated control. Note thinned skin, ruffled fur, and scarce hair of treated mice.

Table 1. The effect of age and dose on the induction of fatal wasting disease by cortisol acetate in C3H mice.

Age at injection (days)	Dose of cortisol acetate		Mice dying of wasting disease	
	Total (mg)	Body weight (mg/g)	No. of mice*	Mean survival time (days after injection) ±S.E.
1	0.1	0.06	7/10	12.4 ± 0.4
	.25	.16	19/19	10.6 ± 0.7
5	.1	.03	5/8	13.0 ± 1.4
	.25	.07	21/32	14.0 ± 0.9
7	.5	.12	4/4	9.8
10	.5	.12	6/6	22.3 ± 2.8
	1.0–1.4	.25	11/11	8.0 ± 0.4
14	0.5	.08	0/7	
	1.5–1.75	.25	5/11	10.6 ± 0.7
21	0.5	0.07–0.1	1/13	16
	1.25–1.5	.25	7/11	14.8 ± 2.7
28–35	2.5–3.25	.25	3/12	17.3
>70	4.5–5.5	.25	0/11	

* Numerators are the number of mice dying of wasting disease; denominators are the number of mice injected.

drome. The growth of the treated animals was noticeably impaired in comparison with noninjected littermates by the 3rd day after drug administration. The skin was thinned and wrinkled and hair growth was markedly impaired with the final appearance of a ruffled, scarce fur coat. Diarrhea, often of the hemorrhagic type, appeared about a week after the injection. All the animals in this group died within 6 to 15 days of the injection (Table 1).

With decreasing doses or increasing age of the animals at the time of injection, the course of the disease was prolonged and less severe (Fig. 1). There was emaciation and muscular atrophy of the animals, associated with a high-stepping gait. Some of the mice recovered after a variable period of stunted growth. The effect of age on the susceptibility to cortisol acetate was particularly obvious when the dose was increased to 0.25 mg/g of body weight. While this dose resulted in a fatal wasting disease in all of the mice up to 10 days of age at the time of injection, it had an irregular effect on mice 14 to 21 days old, and failed to produce a fatal disease in mice that were 10 weeks old at the time of injection (Table 1).

Pathological changes included marked reductions in the weights of the thymus and spleen, accompanied by increases in the weights of the liver, kidneys, and heart. Hemorrhages were often present in the small intestine and foci of calcification were occasionally seen in the myocardium. In no case did we observe overt signs of infection that may have led to the death of the animals. With remission of the disease, the weights of the various organs returned to normal. Adult mice treated with cortisol showed a profound reduction in the weight of the thymus similar to that found in suckling mice. However, the weight of the spleen in young mice treated with cortisol was less than one-fifth that of the controls, whereas the spleen in adult mice treated in the same way was approximately one-half that of the control mice.

The administration of large doses of adrenal corticosteroids to animals produces severe metabolic derangements associated with protein breakdown and increased gluconeogenesis resulting in a negative nitrogen balance, cessation of growth, muscle wasting, and thinning of the skin (*8*). The wasting effect is accentuated in newborn or young animals, and in these animals a single injection of cortisol acetate is sufficient to inhibit growth for a prolonged period and lead to a fatal wasting disease.

Atrophy of the thymus is apparent within 1 day of injection of cortisol. The drug-induced wasting process is very similar to that seen in anmials thymectomized at birth. Therefore, it is possible that the thymus may have some form of antagonistic action to the adrenal corticosteroids which is lost upon ablation of the thymus or negated by large doses of corticosteroids. Dougherty *et al.* have reported an enzyme system in the thymus of mice which is capable of inactivating cortisol, and which is itself stimulated upon administration of this drug (*9*). Recent work has shown that if the thymus of newborn animals is enclosed within cell-impermeable chambers and is then implanted into mice thymectomized at birth, the lymphoid population reappears (*10*). This effect may be due to a lymphoid stimulating humoral factor (*10*) or possibly to an antagonistic effect of the thymus tissue to adrenal cortical hormones.

The destruction of the thymus in runt disease (*2*) may be due either to a graft versus host reaction, or to an increased adrenal cortical hormone level resulting from a graft-host interaction. In the absence of the thymus, corticosteroids produced by the adrenals may be sufficient to maintain the runt disease. Adrenalectomy has been shown to ameliorate the wasting disease induced by injection of parental lymphoid cells into irradiated hybrid mice (*11*).

The effect of age on the susceptibility of the animals to cortisol acetate parallels the susceptibility of nonirradiated F_1 hybrid hosts to wasting disease resulting from the injection of parental lymphoid cells (*12*). It is possible that this age effect reflects either a decreased sensitivity of lymphoid structures to corticosteroids or an increased capacity of the animal to catabolize adrenal cortical hormones.

MICHAEL SCHLESINGER
RAYMOND MARK
Immunogenetics Research Unit,
Duke University Medical Center,
Durham, North Carolina

References and Notes

1. P. A. Gorer, J. F. Loutit, H. S. Micklem, *Nature* **189**, 1024 (1961).
2. R. E. Billingham and L. Brent, *Phil. Trans. Roy. Soc. London, Ser. B* **242**, 439 (1959); G. W. Siskind and L. Thomas, *J. Exptl. Med.* **110**, 511 (1959).
3. J. J. Trentin, *Federation Proc.* **17**, 461 (1958); H. S. Kaplan and B. H. Rosston, *Stanford Med. Bull.* **17**, 77 (1959).
4. J. J. Trentin, *Proc. Soc. Exptl. Biol. Med.* **92**, 688 (1957); O. Vos, M. J. de Vries, J. C. Collenteur, D. W. van Bekkum, *J. Natl. Cancer Inst.* **23**, 53 (1959).
5. J. F. A. P. Miller, *Proc. Roy. Soc. London, Ser. B* **156**, 415 (1962); D. M. V. Parrott, *Transplant. Bull.* **29**, 102 (1962).
6. D. M. V. Parrott and J. East, *Nature* **195**, 347 (1962); D. W. H. Barnes, J. F. Loutit, H. S. Micklem, *Ann. N.Y. Acad. Sci.* **99**, 374 (1962).
7. T. F. Dougherty, *Physiol. Rev.* **32**, 379 (1952); ———, in *The Lymphocyte and Lymphocytic Tissue* (Hoeber, New York, 1960), p. 112.
8. V. di Raimondo, in *The Adrenal Cortex*, (Hoeber, New York, 1961), p. 70.
9. T. F. Dougherty, M. L. Berliner, D. L. Berliner, *Endocrinology* **66**, 550 (1960).
10. R. H. Levey, N. Trainin, L. W. Law, *J. Natl. Cancer Inst.* **31**, 199 (1963); D. Osoba and J. F. A. P. Miller, *Nature* **199**, 653 (1963).
11. H. S. Kaplan and B. H. Rosston, *Transplant. Bull.* **6**, 107 (1959).
12. W. G. Fiscus, B. T. Morris, Jr., J. Session, J. J. Trentin, *Ann. N.Y. Acad. Sci.* **99**, 355 (1962).
13. We are grateful for the encouragement and advice of D. B. Amos. This study was supported in part by a U.S. Public Health Service international postdoctoral fellowship No. FF-547 (M.S.) and by U.S. Public Health Service research grant No. CA-07371-01 (R.M.).

5 November 1963