extract. The effects of increasing the concentration of the insoluble collagen substrate from 0 to 20 mg/ml were also studied at *p*H 5.5 and 32°C, with 5 mg per milliliter of activated extract. In similar fashion, the effect of varying the time, *p*H, and temperature of incubation on the percentage of insoluble hydroxyproline from insoluble collagen (10 mg/ml) by activated extract (5 mg/ml) was studied. More than half of the hydroxyproline solubilized in these experiments was dialyzable.

These results (Tables 2 and 3) demonstrate (i) an essential linearity of response with enzyme concentration; (ii) "excess substrate inhibition" at concentrations exceeding 15 mg of insoluble collagen per milliliter; (iii) a linear increase in collagenolysis with temperature, and approximate doubling of enzyme activity with each increment of 10°C; (iv) a *p*H optimum of about 5.5; (v) and that the bulk of the reaction was completed at *p*H 5.5 within 4 hours. With respect to properties (ii) and (iv), the collagenolytic activity of the connective tissue appears similar to that described for pancreatic collagenase (*8*).

Finally, in separate experiments, the activity of both necrotic and intact rat skin on insoluble collagen was not inhibited by 0.01*M* EDTA, 0.001*M* *p*-chloromer-curibenzoate, or soybean trypsin inhibitor (2 mg/ml). Almost all of this collagenolytic activity was inhibited by 0.05*M* phosphate buffer, however.

These results suggest that both necrotic and intact connective tissue contain a protein component which, after limited proteolytic activation, breaks the primary peptide backbone structure of collagen. This collagenolytic activity of the connective tissue was not inhibited by a mercuric salt; nor was it associated with any general proteolytic activity. It therefore appears that this collagenolytic activity could not result from general tissue cathepsin activity, but rather was relatively specific for collagen. Finally, since this collagenolytic activity was not inhibited by EDTA, it could not have resulted from bacterial collagenase contamination of these tissue extracts (*13*).

It is difficult to explain why the necrotic wound should not contain any "activated" collagenolytic activity. Perhaps the activated enzyme was digested by the various peptidases which presumably exist in necrotic wounds, and was thus destroyed, or perhaps it was rapidly drained away from the area of the necrotic wound into the circulation along with the breakdown products of collagen. Both of these explanations seem possible, but, without supporting data, they remain speculative.

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Immunity and Susceptibility toward Cheek Pouch Transplants of a Mouse Leukemia

Abstract. *Intravenous sensitization with spleen or leukemic cells of mice and certain rats immunized Syrian hamsters toward cheek pouch heterografts of a mouse leukemia. By contrast, sensitized hamsters given cortisone became more susceptible to the challenging leukemic graft. Neither response was elicited by sensitization with cells of the guinea pig, Syrian hamster, rabbit, or human being.*

Heightened immunity toward cheek-pouch heterografts of a mouse leukemia, AK-4, can be induced in the Syrian hamster by prior exposure of the hamster to the leukemic cells or to normal AKR spleen cells. By contrast, administration of cortisone with the leukemic cells or spleen cells results in increased susceptibility. Cortisone alone does not have this effect.

Increased susceptibility evoked in this way resembles immunologic enhancement in some respects, but appears to be dependent upon some special property of the cheek pouch, which is not yet fully understood. Immunity to AK-4 heterografts, on the other hand, is much less site-specific and can be induced by exposing the hamster to cells from strains of mice other than AKR (*1*).

The present experiments (*2*) were done to determine whether susceptibility can be similarly induced by exposing the cortisone-treated hamster to out-of-strain mouse cells, and whether sensitization with sufficiently "foreign" cells fails to result in either susceptibility or immunity.

The results of these experiments indicate that both susceptibility and immunity are not only specifically induced states, but are probably related immunologic states, since their specificity is approximately the same. Neither state is evoked by sufficiently "foreign" sensitizing cells; and which immunologic behavior is evoked by less "foreign" cells appears to depend upon whether or not the hamster is exposed to cortisone at the time of exposure to antigen.

The sensitizing out-of-strain or "foreign" (Table 1) cells were taken from the normal spleens or experimental neoplasms of four strains of mice, Wistar or Sprague-Dawley rats, guinea pigs, and rabbits. Cells were also obtained from a human leukemic spleen (*3*), and from the normal spleens of Syrian hamsters of the same closed but not pure-bred colony which provided the experimental subjects (*4*). For the sensitization, 1.0×10^7 of these unwashed, dissociated spleen or leukemic cells were injected intravenously into different groups of hamsters by retro-orbital puncture. Four weeks after sensitization all of the hamsters were challenged with 1.0×10^7 AK-4 leukemic cells implanted into the right cheek pouch. Part of each group was treated with cortisone (*5*) at the time of sensitization, and cortisone treatment was continued to the end of the experiment, usually 3 to 4 weeks after challenge.

Criteria for evaluating the subsequent behavior of the challenging AK-4 cells inoculated into the cheek pouch, were

as follows: (i) Temporary growth of the inoculum, the normal fate of AK-4 grafts in the cheek pouches of unsensitized hamsters, was taken to indicate no preexisting immunity. Such transient growth was marked by the appearance in the challenged cheek pouch of small, vacularized tumors by 5 to 7 days, which regressed completely by 10 to 14 days. (ii) Immunity (accelerated rejection of the challenging grafts) was characterized either by complete failure to implant (50 percent of pouches), or by the development of nonvascular and barely measurable nodules which regressed completely by 7 to 10 days. (iii) Susceptibility was evidenced by progressive growth of the challenging inoculum beyond 14 days. Although such tumors underwent necrosis, ulceration, adhesion, and (occasionally) regression, death of the host with viable cheek-pouch tumor was by far the most frequent observation. There was often histological evidence of local infiltration, but there was no evidence of disseminated leukemia.

In general (Table 1), the murine cells examined—spleen cells or leukemic cells of AKR, C3H, C57B1/6, LAF_1 mice, and Wistar rats—were capable of inducing immunity in unconditioned hamsters, or susceptibility in cortisone-conditioned hamsters. On the other hand, the cells of the nonmurine species examined—rabbits, guinea pig, human, and Syrian hamster—were not only incapable of immunizing but were also incapable of inducing susceptibility. Thus, depending on their origin, the same cell suspensions were capable of inducing both immunity and susceptibility, or neither. These observations suggest (i) that increased resistance is not, in fact, a nonspecific reaction to foreign tissue, but rather reflects a specific acquired immunity to graft antigens possessed in common by the leukemic and normal spleen cells of certain murine species; and (ii) that increased susceptibility, thus linked to increased resistance, may be a cortisone-dependent variant response to the same antigens. The antigen or antigens in question would appear to be absent (or in insufficient concentration) in the tissues of the nonmurine species examined. The results with Sprague-Dawley cells constitute the single, perhaps not unimportant, exception to the relatedness of immunity, susceptibility, and the murine origin of the sensitizing cells. Though murine, Sprague-Dawley cells were incapable of immunizing, but they were, nevertheless, capable of inducing susceptibility in the presence of cortisone.

Table 1. Induction of heightened susceptibility or immunity toward a challenging inoculation of 1.0×10^7 AK-4 cells in the cheek pouch of the hamster 4 weeks after intravenous sensitization with 1.0×10^7 spleen or leukemic cells. Symbols in parentheses denote strain-specific leukemias used as source of sensitizing cells.

Strain	Sensitizing cells			
	Susceptibility with cortisone*		Immunity without cortisone†	
	Spleen	Leukemic	Spleen	Leukemic
Mouse				
AKR (BW5147)	7/11‡	6/15	6/6‡	8/8
C3H (E9514A; 6C3HED)	13/25	9/16	15/15	22/22
C57B1/6 (C-1498)	12/16	5/8	9/9	4/4
LAF_1	11/18		16/16	
Rat				
Wistar (WR-3)	3/10	7/16	5/9	2/10
Sprague-Dawley	5/12		0/8	
Rabbit				
	0/7		0/8	
Guinea pig				
	0/19		0/15	
Human				
		0/18		0/18
Syrian hamster				
	0/15		0/9	

* Ratio of the number susceptible to the number challenged. † Ratio of the number immune to the number challenged. ‡ Six sensitizing inocula of spleen cells. See text.

The mechanism by which cortisone changes the specific immunologic response from one resulting in transplantation immunity to one resulting in increased susceptibility toward a heterografted leukemia is incompletely understood. What is known thus far is that the susceptible state is passively transferrable (*1*), indicating that antibody may indeed be present in the cortisone-conditioned, sensitized hamster. Such findings suggest that cortisone may have altered, rather than entirely suppressed, the antibody response. In this light, cortisone-imposed susceptibility might be regarded perhaps as a kind of drug-induced immunologic enhancement, in which the immunosuppressant drug functions to diminish the antibody titer. As such, the results would differ little from those of Boyse *et al.* or Möller *et al.* (*6*), in which enhancement is related to small quantities or appropriate dilutions of isoimmune serums. These experiments do not exclude the possibility that cortisone may impose qualitative changes in the hamster's antibody response as well as, or instead of, quantitative changes. The results with Sprague-Dawley cells bring up the further complicating possibilities that two kinds of related but separate antigens ("immunizing" and "enhancing") may take part, or that the dose of antigens together with kinetics of the immune response as affected by cortisone are the determining factors.

Whatever the ultimate solution of this problem, the profound influence of cortisone in altering the immune response is further indicated (Table 1) by the observations on hamsters given six sensitizing inoculations of AKR spleen cells. The additional sensitization completely suppressed any implantation of the challenging inoculum (100 percent of pouches). In cortisone-conditioned hamsters, however, six sensitizing inoculations resulted in the same degree of susceptibility that is normally evoked by a single inoculum. Thus, although the dose of antigen may influence the degree of the immune response, the effect of cortisone appears to be the major determinant in the induction of susceptibility (*7*).

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