complex formation. This can be achieved not only by precipitating catalase by anticatalase IgG-preparation, but to the same extent also by Fab-fragments forming a soluble complex with the catalase mutant. Analogous experiments with essentially the same result were performed at 37 °C and 52 °C, indicating that this stabilizing effect can be observed in a rather broad temperature range[16]. Furthermore, in order to verify the specificity of the stabilizing effect of anticatalase, the same type of experiment was performed using an anti-carbonic anhydrase B-IgG-fraction and its corresponding Fab-preparation instead. These antibodies, prepared by the same procedure, were used in the same concentration range. In this control experiment no stabilization effect could be observed[16]. In another series of experiments the effect of the antigen-antibody ratio was investigated. Using the anticatalase IgG-preparation, a lower catalase activity is observed in the zone of antigen-antibody equivalence. This interference is no longer observed if a moderate excess of antibody (2.5 molar ratio) is used. No such effect could be detected when the Fab-preparation was used as a stabilizing agent.

Discussion. Catalase is one of the few enzymes retaining full enzymatic activity when bound in an antigen-antibody complex. This complex formation stabilizes the active conformation of the enzyme, without interfering with its active site. The effect is particularly striking in a labile enzyme variant crossreacting with the antibody specific for its normal counterpart. The stabilization of a human catalase mutant is essentially the same phenomenon as that observed by FEINSTEIN et al.[8,9] in acatalasemic mice. This enzyme defect resembles that observed in Swiss type acatalasemia in as much both are due to the synthesis of an unstable enzyme variant. In both instances insolubilization is not a requisite of stabilization. As shown here Fab-fragments are as efficient as the IgG-preparation in stabilizing the labile catalase mutant.

This stabilization effect by complex formation lends further support to the assumption that the fixation of a particular conformation of the subunits counteracts the disintegration of an oligomer-enzyme. It may be anticipated that this action is especially pronounced if the interacting forces are altered as a result of a mutation. Therefore, it is probable that the mechanism of stabilization of variant catalase resembles that of β-D-galactosidase in *E. coli*, where, even with an inactive enzyme from a mutant strain full activity could be restored by means of antibodies specific for the wild type enzyme[6].

These observations are of theoretical as well as of practical interest. On one hand, the preparation of active antigen-antibody complexes of labile enzyme variants may provide a valuable tool for their determination and isolation. On the other hand, experimental evidence has been presented recently by FEINSTEIN[17] that upon injection of catalase antibodies to acatalasemic mice it seems possible to counteract the inactivation of labile enzyme variants in vivo also. Although this preliminary result does not permit an extrapolation, the idea of an in vivo stabilization of labile mutants deserves further consideration[18].

Zusammenfassung. Die instabile Katalase-Variante, welche in den Erythrocyten des Akatalasie-Falles A.B. vorkommt, lässt sich in vitro durch Zusatz von Anti-Katalase IgG-Fraktion oder von Fab-Fragmenten ohne Aktivitätseinbusse stabilisieren. Es wird angenommen, dass dies auf eine Fixierung der aktiven Konformation des Enzyms zurückzuführen ist.

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Immunochemical Relationships Among Lysosomal Acid Phosphohydrolases: DNase II and Nonspecific Phosphodiesterase

Among the lysosomal enzymes are a group of acid phosphohydrolases. These include both phosphomono- and diesterases. All chromatograph on CM-cellulose[1], and it has been reported that DNase II possesses activity against *bis-p*-nitrophenyl phosphate in addition to its nucleolytic activity[2,3]. These findings of common location and certain closely related chemical properties led us to conjecture that lysosomal acid phosphohydrolases may have arisen by the processes of gene duplication and mutation from a common ancestral, rather nonspecific phosphohydrolase. Proteins with common origins often have the same antigenic determinants[4]. For this reason we have started an immunological comparison of the acid phosphohydrolases. We report here a comparison of the immunoprecipitation reactions of cow and hog spleen DNase II and hog spleen nonspecific phosphodiesterase (PDE) with rabbit anti-hog spleen antiserum.

For these experiments the enzymes were purified as previously described for beef spleen DNase II[5]. After chromatography on CM-cellulose and Sephadex G-100 the specific activities of the enzymes in units/mg were: Beef spleen DNase II, 216; hog spleen DNase II, 68; hog spleen phosphodiesterase, 1.71. Antisera were prepared in New Zealand white rabbits by immunization with 3 mg of purified enzyme in Freund's complete adjuvant followed twice at weekly intervals by injection of 3 mg of enzyme in incomplete adjuvant. Blood was obtained after the 4th week. IgG was prepared by ammonium sulfate fractionation and chromatography on DEAE cellulose[6].

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Comparison of DNase II and phosphodiesterase by immunoprecipitation

	Enzyme (μg/ml)	Antibody or control									
		Saline		Normal rabbit		Cow DNase		Hog DNase		Hog PDE	
		U/ml[a]	A_{280}[b]	U/ml	A_{280}	U/ml	A_{280}	U/ml	A_{280}	U/ml	A_{280}
A)	Bovine DNase II										
	0	0	0	0	0	0	0	0	0	0	0
	10	75	0	86	0	0	55	32	16	55	7
	40	216	0	302	0	29	108	175	8	250	12
	80	612	0	574	0	141	137	394	5	499	10
	120	944	0	980	0	435	123	724	4	826	12
	200	1475	0	1458	0	1007	102	1350	5	1419	12
B)	Hog DNase II										
	0	0	0	0	0	0	0	0	0	0	0
	10	80	0	106	0	0	8	0	76	39	15
	40	250	0	284	0	15	2	0	146	136	10
	80	428	0	416	0	166	0	46	188	330	6
	120	630	0	636	0	380	0	178	160	458	0
	200	972	0	1001	0	727	0	578	134	691	0
C)	Hog phosphodiesterase										
	0	0	0	0	0	0	0	0	0	0	0
	10	0.8	0	1.1	0	0.3	1	0.5	4	0	30
	40	2.8	0	3.1	0	1.7	25	2.1	17	0.1	93
	80	7.3	0	6.5	0	4.8	12	4.6	0	0.8	141
	120	9.6	0	9.8	0	7.6	0	8.0	0	3.1	112
	200	16.5	0	15.9	0	14.1	0	14.8	0	9.1	56

[a] Units/ml $\times 10^2$; [b] $A_{280} \times 10^3$

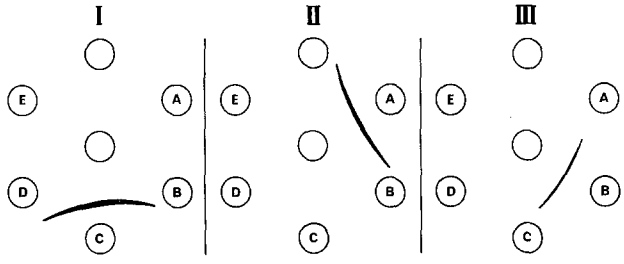

Fig. 1. Immunodiffusion of porcine and bovine spleen DNase II and porcine phosphodiesterase against homologous antisera. The antigen is in the center well and the rabbit antisera or saline in the peripheral wells as follows: A, antiporcine spleen DNase II; B, antibovine spleen DNase II; C, antiporcine phosphodiesterase; D, normal rabbit serum; E, saline. The center wells contain: I, porcine spleen phosphodiesterase; II, porcine spleen DNase II; III, bovine spleen DNase II.

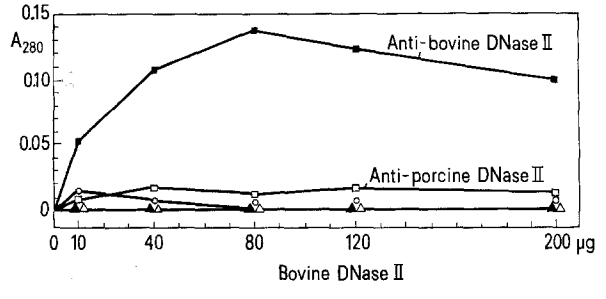

Fig. 2. A_{280} of dissolved precipitate following treatment of bovine DNase II with rabbit antibovine DNase II IgG (■), antihog DNase II IgG (□), antihog phosphodiesterase IgG (○), normal rabbit IgG (▲) or saline (△).

Evidence for cross reactivity was sought first by double diffusion in agar. Despite variation of the antigen concentration from 0.1 to 50 μg/ml and use of a 2-, 5- or 10-fold diluted serum the only precipitin lines obtained were in the homologous reactions (Figure 1).

For the immunoprecipitation reactions, 0 to 200 μg of antigen was diluted with borate-buffered saline, pH 8.0 (BBS) to 0.5 ml. Specific IgG (7 mg of protein in 0.5 ml BBS) was added and the tube mixed. Precipitation occasionally followed mixing. The tubes were incubated at 37° for 30 min and at 4° for 8 h. The precipitates were collected by centrifugation at low speed, washed in cold BBS and dried. The supernatant solutions were saved for enzyme assays. After solution of the precipitates in 0.5 ml of 1 *M* NaOH the A_{280} of each was determined. Each reaction was performed in triplicate. The precipitation data obtained with bovine DNase II is shown in Figure 2 and the residual enzyme activity in Figure 3. The data for all the experiments are summarized in the Table.

The data from the residual activity experiments were submitted to logarithimic transformation and a two-way analysis of variance. With bovine DNase II as antigen, no significant difference ($P \leq 0.05$) at any concentration was apparent in residual activity after treatment with either saline or normal rabbit IgG. There were significant differences among the responses to the other antibodies, and between these and normal rabbit IgG or saline at all concentrations of enzyme except 200 μg. At this level only antibovine DNase II IgG gave results significantly different from the remainder. The homologous reaction (bovine DNase II-antibovine DNase II) was always significantly lower than the others. When hog DNase II was the antigen, the results after treatment with saline and normal rabbit IgG were significantly different only

at the 0 and 10 μg levels. There were significant differences among all the reactions at 10 and 40 μg, except for the results with antibovine and antiporcine anti-DNase II IgG, as treatment with either of these resulted in no activity in the supernatant at the 10 μg level. At the higher levels all the reactions (except those with saline and normal rabbit IgG aforementioned) led to significant differences. Here, too, the homologous reaction always resulted in residual DNase levels lower than the rest. The results with the porcine phosphodiesterase were similar although not as clear cut. The homologous reaction was always lowest, but significantly so only at the 0, 10 and 40 μg levels. In general, the differences among the other reactions were not significant.

Linear regression analysis was also carried out on the data for residual activity using the levels 40, 80, 120 and 200 μg. The linear correlation coefficients varied from 0.97 to 0.999.

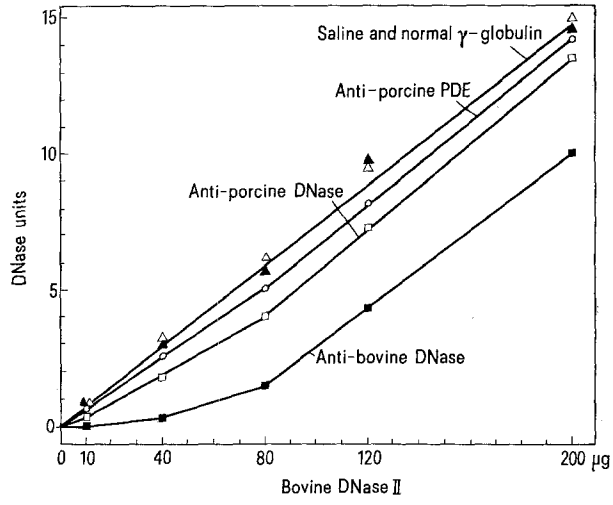

Fig. 3. Residual enzyme activity after precipitation of bovine DNase II with rabbit antibovine DNase II IgG (■), antihog DNase II IgG (□), antihog phosphodiesterase IgG (○), normal rabbit IgG (▲) or saline (△).

It is obvious from both the precipitation and residual activity data that the strongest reactions occurred between a particular enzyme and the antibody to it. There was considerable cross reactivity between the DNases from the 2 different sources. There was some cross reaction between the hog spleen DNase and phosphodiesterase and little between the bovine spleen DNase and hog spleen phosphodiesterase.

These data bear out the close relationship between DNase II from 2 different species, a conclusion reached by CORDONNIER and BERNARDI on the basis of other types of studies[7]. The data also indicate that there is some slight antigenic relationship between hog spleen DNase II and phosphodiesterase, a point not obvious from our studies with antibody affinity columns[8]. However, the antigenic relationship between DNase II and nonspecific phosphodiesterase from one species (hog) is even less than that between the DNases II from two distinct species (hog and ox), and whether this betokens an evolutionary relationship or not remains to be seen.

Zusammenfassung. Immunopräcipitationsreaktionen mit Schweinemilz, Desoxyribonuklease II, unspezifische Phosphodiesterase und Rindermilz-DNase II zeigen, dass eine engere immunologische Beziehung zwischen den Nukleasen beider Spezies besteht, als zwischen den beiden Schweinephosphohydrolasen.

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Raster-elektronenmikroskopische Untersuchungen an der Hausstaub- und Asthma-Milbe *Dermatophagoides pteronyssinus* (Trouessart, 1897) (Acarina: Astigmata)

1897 beschrieb TROUESSART[1] zum erstenmal die Milbe *Dermatophagoides pteronyssinus* unter dem Namen *Mealia pteronyssina*. 1953 ordnete DUBININ[2] diese Art der Gattung Dermatophagoides Bogdanov, 1864 zu.

1964 fanden VOORHORST und das Biologen-Ehepaar SPIEKSMA[3], dass der grösste Teil der Milbenfauna im Staub der Häuser aus *Dermatophagoides preronyssinus* besteht. Später ist es ihnen gelungen, diese Art zu züchten und mit den Extrakten Hautteste durchzuführen. Da die meisten Patienten auf Extrakte von *Dermatophagoides pteronyssinus* und von Hausstaub gleich reagierten, schlossen sie daraus, dass diese Milbe mit dem Hausstauballergen identisch und somit für Asthma bronchiale, Rhinitis u.a. verantwortlich sei.

1966 wurde die Morphologie der Tiere erstmals gründlich von FAIN[4] beschrieben. 1967 wurde die Milbe wegen ihres häufigen Vorkommens in Häusern von SPIEKSMA[5] als «Hausstaubmilbe» benannt. 1970 publizierte WHARTON[6] die erste REM-Aufnahme einer Tritonymphe. 1971 konnten GRIFFITHS et al.[7] *Dermatophagoides farinae* von *Dermatophagoides microceras* mit Hilfe des REM trennen. 1971 beschrieb GRIFFITHS[8] die Gefriertrocknung als eine geeignete Fixationsmethode für verschiedene weichhäutige Acarina.

Material und Technik. Die Tiere stammen aus den Zuchten eines der Autoren[9]. Unsere Untersuchungen begannen

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