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Serum Enzyme Levels in Lines of Chickens Differing in Susceptibility to Leucosis.* (29208)

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Genetic differences in susceptibility to leucosis in chickens are well established. The purpose of this study is to find out if the chemical basis for such differences is manifested in differences in the level of enzymes in serum. It is recognized, however, that there are many other possibilities besides enzyme level which might be responsible for differences in susceptibility to leucosis.

The stocks employed were a resistant and a susceptible line developed at Cornell University(1). These lines differ markedly in mortality resulting from leucosis, especially the neural form. Studies were conducted at an early age because of evidence indicating the first fortnight posthatching to be a critical period in exposure to the disease(2). Only females were used since they show greater mortality from the disease than do males(3). Enzymes studied were of a wide variety. Their selection was based partially on the availability of routine assay methods. Leucosis is caused by viruses(4), therefore protease and nuclease assays were included, as such enzymes might conceivably inactivate virus either in the vascular system or in the organ(s) from which the serum enzymes originate.

Materials and methods. A susceptible line and a resistant (K) line in respect to leucosis were obtained as hatching eggs from Cornell University. Chicks were reared separately from adults in batteries; no attempt was made to expose them to leucosis. Blood was sampled from females and serum poured off after incubation at 37°C for 1½ hours. Assays for acid phosphatase were performed on the day blood was sampled; a decline of 20% in serum acid phosphatase activity had previously been observed after storage for one week at —20°C. Deoxyribonucleases I and II were also assayed on fresh serum. Other enzyme assays were performed within 9 days of blood sampling. Serum was stored at —20°C. A separate experiment showed no change in cathepsin activity after one week at —20°C. All other enzymes studied have been previously shown to be stable under conditions of this study.

An incubation temperature of 41°C and a Coleman Universal spectrophotometer were used for all assays except for glutamic-oxalacetic transaminase and lactic dehydrogenase, which were done at room temperature (29-35°C) with a Beckman DU spectrophotometer. Acid phosphatase, alkaline phosphatase, and cathepsin were assayed by the methods of Andersch and Szczypinski(5), Bessey *et al*(6), and Anson(7), respectively. In assays for deoxyribonucleases enzyme activity was computed using a standard curve of known amounts of purified enzyme (Worthington). Deoxyribonuclease II activity was measured essentially by methyl green method of Kurnick and Sandeen(8), and deoxyribonuclease

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TABLE I. Serum Enzyme Levels of 10-day-old Females from Lines Susceptible and Resistant to Leucosis.

Enzyme	Units	Enzyme activity* Susceptible line	Resistant line
Acid phosphatase	mM nitrophenol/1/hr		
1st exp		.90 ± .07	1.24 ± .11
2nd "		1.20 ± .09	1.23 ± .09
Alkaline phosphatase	" " "	147 ± 19	187 ± 44
Amylase	Somogyi units/100 ml	1594 ± 62	1707 ± 125
Cathepsin	meq × 10^{-4} tyrosine		
1st exp		1.14 ± .09	1.85 ± .12
2nd "		1.62 ± .28	2.16 ± .07
Deoxyribonuclease I (alkaline)	μg/ml	.57 ± .10	.81 ± .08
Deoxyribonuclease II (acid)	" "	32.6 ± 2.8	29.5 ± 3.4
Glutamic-oxalacetic transaminase	units/ml	314 ± 10	282 ± 14
Lactic dehydrogenase	" "	866 ± 79	966 ± 66
Leucine aminopeptidase	G-R units	1890 ± 150	1985 ± 117
Lipase	Sigma-Tietz units/ml	.46 ± .13	.32 ± .05

* Mean ± standard error. Ten birds per line, except 12 per line for acid phosphatase (2nd exp) and DNase II, and 5 per line for cathepsin (2nd exp).

I by a similar method using alkaline conditions. For the latter, 0.4 ml of serum was added to 6 ml substrate solution in water bath at 41°C. Substrate consisted of 1 mg DNA-methyl green per 5 ml of 0.05 M Tris-HCl buffer, having a pH of 7.5 and containing 0.0075 M $MgSO_4$. After 4 hours' incubation 1 ml 0.33 M sodium citrate was added, and O.D. read at 640 mμ after standing overnight at room temperature. This reading was subtracted from that of an unincubated control.

Amylase, lipase, and leucine aminopeptidase were analyzed using reagents and directions supplied by Sigma Chemical Co., St. Louis, Mo., according to Technical Bulletins Nos. 700, 800, and 250, respectively; these assays are based on the methods of Somogyi (9), Tietz *et al*(10), and Goldbarg and Rutenburg(11), respectively. Glutamic-oxalacetic transaminase and lactic dehydrogenase were analyzed using reagents and directions supplied by Worthington Biochemical Corp., Freehold, N. J.; these assays are based on the methods of Karmen(12) and Wacker *et al*(13), respectively.

Pooled serum samples from 5 birds were used for lipase assays. All other assays were performed as single determinations of individual sera. Certain enzymes were not assayed for because preliminary studies showed them to be absent from serum of young chickens. These were chymotrypsin, glutamic dehydrogenase, ornithine carbamyl transferase, and trypsin. Ribonuclease was excluded from the study because activity was either very low or nil in several preliminary assays. Cathepsin was chosen over pepsin because its activity was twice as great.

Statistical analysis was by analysis of variance. Logarithms were used for alkaline phosphatase, since variance tends to vary directly with the mean.

Results. Average enzyme levels of 10-day-old chicks are given in Table I. These levels are about 2 to 3 times greater than the levels at the youngest age studied by McDaniel and Chute(14) for the phosphatases, lactic dehydrogenase, and glutamic-oxalacetic transaminase, using methods very similar to those employed in the present study. The level of deoxyribonuclease I is also considerably higher than the value reported by Kurnick (15) using 2 serum samples from chickens of unstated age. There are no previously reported values for the serum level of the remaining enzymes in chickens. It is noteworthy that the levels of both amylase and

TABLE II. Serum Enzyme Levels of 4-week-old Females from Lines Susceptible and Resistant to Leucosis.

Enzyme	Units	Enzyme activity*	
		Susceptible line	Resistant line
Cathepsin	meq $\times 10^{-4}$ tyrosine	.97 ± .10	1.55 ± .12
Deoxyribonuclease I	μg/ml	.40 ± .06	.46 ± .07

* Mean ± standard error. 22 birds per line for cathepsin and 15 birds per line for DNase I.

leucine aminopeptidase are 10 times that of the upper limit of the normal range in humans. Lipase activity is within the normal range for humans.

The level of acid phosphatase was higher in the resistant line than the susceptible line in the first experiment. This difference was statistically significant ($P<.05$). However, in a second experiment the difference between lines was small and not significant. The level of cathepsin was also higher in the resistant line than in the susceptible line. This difference was statistically significant in the first experiment ($P<.01$), and approached significance in the second experiment ($P<.10$). A rather large difference in means was observed with deoxyribonuclease I; this difference only approached significance ($P<.10$). The remaining enzymes exhibited small differences between the two lines. In the case of glutamic-oxalacetic transaminase the difference approached significance ($P<.10$).

Enzyme analyses were also performed at 4 weeks of age on another group of birds. These were done to determine if the differences previously observed in cathepsin level were also present at a later age. Deoxyribonuclease I assays were included because of the suggestive results at 10 days of age. Data are given in Table II. Cathepsin level was higher in the resistant line than the susceptible line. This difference was statistically significant ($P<.01$). A small, nonsignificant difference in lines was found in deoxyribonuclease I level.

Discussion. In comparisons of 2 lines differing in susceptibility to leucosis cathepsin was the only enzyme studied in which a difference was repeatedly observed in its level in serum. The level in the resistant line was 50% higher at 10 days and 60% higher at 4 weeks of age than that of the susceptible line. Further work is needed to clarify whether this difference is in fact associated with leucosis susceptibility, or merely represents a separate difference between these lines unrelated to their differences in mortality from leucosis. An involvement of cathepsin in leucosis susceptibility offers an attractive hypothesis since the enzyme is important in destruction of protein by the cell, and protein is a constituent of virus. Any such involvement of a serum enzyme would not be novel, since evidence has recently been advanced that L-asparaginase in rodent serum possesses leukemia-inhibiting properties(16).

It should be noted that differences have been observed in level of phosphatases in plasma of chickens which had been inoculated with lymphomatous liver filtrate and which subsequently had visceral leucosis(17). However, the conditions were different from those of the present investigation: genetic differences in leucosis susceptibility were not studied, and the birds were deliberately exposed to the disease. Furthermore, enzyme levels were measured in much older birds than in the present study.

The high level of several of the enzymes studied herein has already been noted. The reason for the discrepancy with previous findings in chickens, as reviewed by McDaniel and Chute(14), is not clear. In the present study most of the assays were performed at the body temperature of the chicken (41°C), which is higher than the temperature usually employed (37°C). The lactic dehydrogenase and glutamic dehydrogenase assays were done at a room temperature somewhat higher than is usual. One would expect higher values at the higher temperatures, except for amylase which was corrected for temperature, and deoxyribonuclease level, which was computed from known amounts of purified enzyme assayed at 41°C. However, the high incubation temperature does not account for the magni-

tude of the differences observed. Variation of enzyme level with age is a possible explanation. The birds used in this study were 10 days old and therefore considerably younger than those used in other studies. This is apparently the case with alkaline phosphatase level, which is markedly elevated at 1 and 2 weeks as compared to 6 weeks of age(17,18). Age differences, as opposed to species differences, might also account for the higher levels of leucine aminopeptidase and amylase obtained in chickens than humans.

Summary. Level of 10 enzymes was measured in sera of 10-day-old females from lines resistant and susceptible to leucosis. The level of cathepsin was significantly higher in the resistant line ($P<.01$); similar results were also obtained at 4 weeks of age. No significant and repeatable differences were observed in the other enzymes.

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Serial Multiplication of an Influenza Virus (NWS) in Certain Human Diploid Cell Strains.* (29209)

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Evidence for the serial multiplication of influenza viruses in continuous aneuploid cell lines has been published in only two reports (1,2). Brief mention has been made of the cytopathic effect of 3 influenza viruses in the WI-1 human diploid cell strain, but evidence of viral multiplication or other experimental details was not provided(3). Evidence is presented here that the neurotropic WS strain (NWS) of influenza A virus can multiply with the production of infective virus in certain diploid cell strains derived from human embryonic lung. The formation of hemagglutinating but noninfective virus following inoculation of other strains of influenza virus will also be described.

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Materials and methods. Viruses. Variants of the Stuart-Harris neurotropic strain of the WS influenza A virus (NWS) and of the S-15 strain of swine influenza virus which had re-