2 The authors wish to thank Dr R.C. Garner for providing aflatoxin-B-guanine adduct, and Dr Dennis H. Hseih for providing aflatoxicol and aflatoxin Q_1.
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Serum proteins of mice with splenomegaly[1]

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Summary. Our earlier serum electrophoretic study in 'lethargic' mutant mice showed that the quantity of protein in 1 band is inversely related to the size of the spleen. In this study, we demonstrate that this protein band almost entirely disappears in mice with splenomegaly following spontaneous skin infection. The results suggest that this serum protein may play a role in regulating growth of lymphoid tissue.

In a study of serum proteins[2] from the 'lethargic' mouse, a neurological mutant[3,4], we noted that a protein band (designated as band 2) showed a quantitative change inversely associated with the growth of lymphoid organs, particularly the spleen. This protein band was quantitatively small when the spleens of the mice were in a stage of rapid growth, and increased in quantity when the spleens decreased in weight[2]. It was suggested that band 2 might contain a protein or proteins which act like a chalone to regulate lymphoid cell proliferation in the spleen.

To further substantiate our hypothesis, sera from other mice which exhibit splenomegaly have been studied electrophoretically using polyacrylamide gels (Bio-Rad Laboratories, Richmond, Cal.). The mice studied show an obvious splenomegaly associated with a skin infection. Etiology of the skin infection is not known; it occurs in approximately 25% of the phenotypically normal mice of our breeding colony over 4 months of age but is very rare in 'lethargic' animals. Mice in the colony have been brother-sister inbred for more than 13 years (over 60 generations). The infection invariably begins as a small superficial lesion over the scapular region. At this early stage, the spleens of the mice appear to be normal in size and weight. The skin lesion gradually extends to other dorsal regions of the body and erodes deeply resulting in sloughing of the fur and skin.(figure 1). Mice with the skin infection usually die in 3 months; however, some of them survive as long as 8 months after the onset of the infection.

Materials and methods. Sera were collected from 13 mice (5 males and 8 females) with normally appearing skin (control group), and 14 mice (5 males and 9 females) with skin infections (infected group) that were present from 2 to 5 weeks before blood collection. The mice were 5–7 months old at the time of sacrifice. Body and spleen weights of the mice at the time of sacrifice are summarized in table 1.

Table 1. Body and spleen weights of mice used for serum electrophoretic analysis

	Control	Infected	Statistical difference*
No. of mice	12	13	
Body weight (g)	36.4±0.3**	35.3± 1.1	NS
Spleen weight (mg)	104.7± 3.6	634 ±147.4	$p<0.001$

* Student's t-test; ** mean ± SE.

Table 2. Percentage of 11 different protein bands from mouse sera separated by polyacrylamide gel electrophoresis

	Protein band (% of total gel OD)										
	1	2	3	4	5	6	7	8	9	10	11
Control group	45.9** ± 2.6	13.1 ± 1.4	4.4 ± 0.2	14.6 ± 1.9	2.8 ± 0.3	4.3 ± 0.3	3.0 ± 0.4	2.6 ± 0.4	1.9 ± 0.3	6.0 ± 1.2	1.4 ± 0.2
Infected group	48.9 ± 5.8	5.0 ± 1.0	5.3 ± 1.1	16.3 ± 3.3	3.0 ± 0.4	4.1 ± 0.5	4.4 ± 0.7	3.6 ± 0.5	2.7 ± 0.4	5.8 ± 0.4	1.0 ± 0.2
Statistical difference*	NS	<0.001	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Student's t-test; ** mean ± SE; NS = no significance.

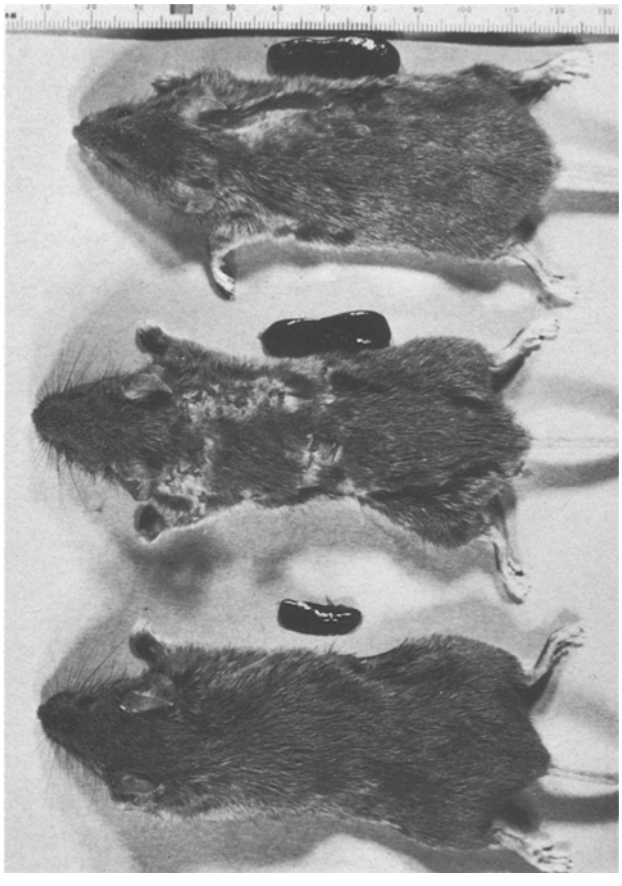

Fig. 1. Mice with their spleens placed beside their bodies. The lower one without skin infection had a spleen weighing 108 mg. The spleens of the other 2 with skin infection over the dorsal regions of their bodies (more severe in the center one) weighed more than 500 mg.

To obtain sera for study, mice were bled by making an axillary pouch and severing the axillary vessels under Nembutal anesthesia. Individual blood samples were placed in small plastic tubes and allowed to coagulate at room temperature for 30–40 min before centrifugation. Sera were then aspirated and stored individually in plastic tubes at −20 °C until the time of electrophoresis.

Polyacrylamide gel electrophoresis was performed using a vertical slab cell Model 221 (Bio-Rad Laboratories, Richmond, Cal.). A 5.5% gel was prepared following the method of Davis[5]. Details of the method were reported in a previous paper[2]. After the completion of electrophoresis, gels were stained with Coomassie blue and destained in methanol-acetic acid. The destained gels were cut into strips containing protein profiles of individual serum samples and were scanned with an UA-5 absorbance monitor (ISCO, Lincoln, Nebraska) at a wavelength of 579 nm. Quantitation of protein bands was accomplished by integrating each peak on the densitometric tracings and converting the peak areas to a percentage of total staining area above background[6].

Protein concentrations in the serum of mice in the control and infected groups were determined using Bio-Rad Assay (Bio-Rad Laboratories, Richmond, Cal.) as described by Bradford[7].

Results and discussion. Results of the protein quantitation from polyacrylamide gels with individual electrophoregrams are summarized in table 2.

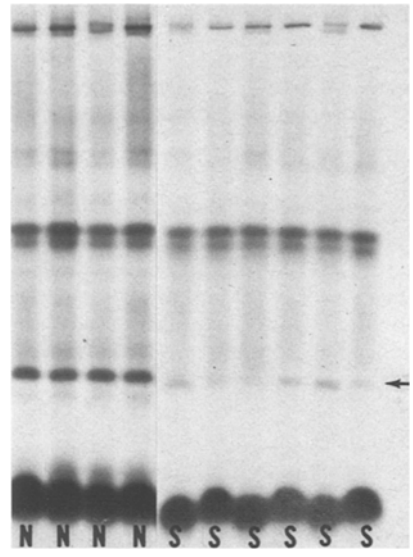

Fig. 2. Serum electropherograms of mice without skin infection (N) and with skin infection (S). Note protein fraction in band 2 (arrow) is diminished in infected group.

As shown in table 2, the only significant difference was seen to occur in band 2. The relative amount of protein(s) in this band 2 was substantially decreased in the skin-infected mice with a splenomegaly (table 1).

Total protein concentrations in the serum of mice of the control and infected groups were 42.8±4.4 and 36.7±5.6 mg/ml serum respectively. Though total serum protein in the infected group was lower than that of the control group, the difference was not statistically significant. Absolute concentrations of proteins in band 2 were calculated from total protein concentrations in the serum; an average of 2.3 µg was found in band 2 for control mice, and 0.7 µg for mice with skin infection.

Results of the present study appear to support our hypothesis that band 2 contains proteins which may behave as a lymphocyte chalone[8,9] to regulate cell proliferation in the lymphoid tissue. We assume that the splenomegaly in the mice with skin infection is due to an increase in lymphoid cell proliferation. To facilitate such cell proliferation, production of lymphocyte chalone which is known to inhibit DNA synthesis[9–12] would have to be turned off as apparently occured in the infected mice used in this study.

1 This study is made possible through support of Medical Research Service of the US Veterans Administration. We thank Dr Thomas B. Pool, Department of Anatomy, The University of Texas Health Science Center, for editorial assistance.

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