

out appreciable effect on intracellular GSH, indicates that GSH-mediated reduction of methaemoglobin⁸, if it occurs at all in the intact erythrocyte, must proceed at an extremely low, and probably physiologically unimportant rate.

Zusammenfassung. Bei der Nachuntersuchung der Nitritwirkung auf das GSH der unversehrten Erythrocyten konnte nur ein geringfügiger Effekt festgestellt werden. Die früher mitgeteilte totale Zerstörung der GSH

wurde auf einen Artefakt zurückgeführt, der während der Eiweissfällung mit Metaphosphorsäure auftrat.

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⁸ D. B. MORRISON and E. F. WILLIAMS JR., *Science* **87**, 15 (1938).

Factors which Influence Normal Values for Serum Lactic Dehydrogenase in Mice

Numerous papers on lactic dehydrogenase (LDH) activity in disease have been published by investigators in both clinical and experimental medicine^{1,2}. Although these, as well as studies on enzyme levels in chicken plasma³ and human serum⁴, have provided extensive data on normal LDH activity in a variety of animal species, little attention has been given to the possibility that intrinsic factors may influence enzyme activity. Of interest is the observation by BIERMAN et al.⁵ that serum LDH levels in children, up to 14 years of age, are higher than in adults.

The objectives of the present study were to determine the effects of sex, age and strain on LDH activity in mouse serum. Concurrently, data were obtained relevant to serum LDH levels in normal mice.

Materials and Methods. Blood was obtained by tail bleeding from mice of 5 inbred strains (A/Fg, C3H/Fg, C57BL/Fg, LCSa/Fg and MOB/Fg). Each strain was represented by 50 animals which were subdivided into 5 age groups (5 males and 5 females per group). Sera were collected by routine laboratory procedures and stored for a maximum of 7 days at -30°C. Serum LDH activity was determined by the method of HENRY et al.⁶ in a Beckman DU spectrophotometer at 340 mμ (Tungsten lamp source). All results were corrected to 32°C.

Results. The effects of sex, age and strain on mouse serum LDH activity were analyzed by means of analysis of variance. Only one significant effect, the interaction of age and strain ($F = 2.05$; $p \geq 0.05$), was found. These findings indicate that mice used in a given experiment on LDH should be from the same strain and approximately the same age. The sex of the animal, by itself or interacting with age and/or strain, does not appear to have a significant effect on mouse serum LDH activity.

The normal ranges and mean values for levels of serum LDH in 5 inbred strains of mice are shown in the Table. The reader is cautioned, however, to use these data as

guideposts rather than absolute figures since it has been suggested that each laboratory, because of variations in procedures, determine its own normal LDH values².

Among the 250 samples tested, 26 sera which did not show hemolysis⁴ were found to have enzyme activities which were significantly elevated. The established ubiquity of polyoma virus in mouse colonies⁷ and recent studies which have demonstrated an increase in LDH activity following the inoculation of mice with filtrates of a variety of murine tumors⁸ suggested a possible explanation for this phenomenon. Sera were obtained from 50 mice and tested, by hemagglutination inhibition procedures, for the presence of polyoma virus antibody. The failure to demonstrate antibody in any of the samples indicates that the elevated serum LDH levels found in our normal mice are not related to infection of these animals with polyoma virus. These findings do not, however, exclude the role of other viruses.

Finally, it should be noted that the 26 samples with increased LDH activities were restricted to animals from 3 of the 5 inbred strains (A/Fg, LCSa/Fg and MOB/Fg). Although no definitive studies have been made, the fact that the LCSa/Fg and MOB/Fg strains originated from a CBAN male × A/Fg female cross and an A/Fg male × CBAN

¹ Ann. N.Y. Acad. Sci. **75**, 1 (1958).

² R. J. ERICKSON and D. R. MORALES, *New England J. Med.* **265**, 478 (1961).

³ L. S. McDANIEL and H. L. CHUTE, *Amer. J. vet. Res.* **22**, 99 (1961).

⁴ R. D. RAPP and E. R. BELL, *Amer. J. clin. Path.* **35**, 116 (1961).

⁵ H. R. BIERMAN, B. R. HILL, L. REINHARDT, and E. EMORY, *Cancer Res.* **17**, 660 (1957).

⁶ R. J. HENRY, N. CHIAMORI, O. J. GOLUB, and S. BERKMAN, *Amer. J. clin. Path.* **34**, 381 (1960).

⁷ W. P. ROWE, J. W. HARTLEY, L. W. LAW, and R. J. HUEBNER, *J. exp. Med.* **109**, 449 (1959).

⁸ V. RILEY, F. LILLY, E. HUERTO, and D. BARDELL, *Science* **132**, 545 (1960).

Normal values for serum LDH in 5 inbred strains of mice

Strain	Number of mice	Age (months)		4-6		7-9		10-12		13+	
		1-3 Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
C3H/Fg	50	950-2050	1450	1200-2650	1850	1100-2450	1550	500-1900	1400	700-2400	1550
C57BL/Fg	50	950-2050	1200	1250-2100	1650	1200-2350	1900	1050-1900	1450	850-2050	1400
A/Fg	50	850-2450	1450	650-3250	1600	800-3300	1400	1000-4200	2550	1350-5350	2750
LCSa/Fg	50	1250-3050	1600	750-2550	1700	700-3700	2150	1350-3250	2200	1150-4250	2050
MOB/Fg	50	850-3650	1550	750-5300	1650	750-4250	1950	600-3850	1600	1200-3250	2350

female cross respectively suggests that levels of serum LDH in normal mice are influenced by genetic factors⁹.

Résumé. Nous avons étudié, chez la souris normale, l'effet du sexe, de l'âge et de la constitution génétique sur

l'activité du sérum LDH. Le seul résultat positif constaté est un rapport entre l'âge et la race. Nous avons établi la portée normale de l'activité du LDH dans cinq races de souris. Son augmentation n'est pas provoquée par une infection due au virus polyome, mais peut être mise en relation avec la constitution génétique de l'animal.

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Immunelectrophoretic Analysis of the Lymph

The lymphatics transport from the tissue spaces not only plasma proteins filtered from the capillaries but also proteins (enzymes, hormones etc.) produced by the tissue cells. The usual procedures of protein fractionation, including the method of paper electrophoresis which is most commonly applied for the separation of protein components of the lymph, do not disclose any significant difference in the composition of blood plasma and lymph¹. The method is apparently not sensitive enough to reveal the presence of minimal quantities of specific proteins formed.

It was supposed that proteins produced in the tissues could be detected in the lymph flowing from the respective organ by immunelectrophoretic analysis.

Mongrel dogs of both sexes, under chloralose general anaesthesia, were used. The cervical and intestinal lymphatic trunks and the main lymphatic channel of the liver were exposed and polyethylene tubes were inserted. In some cases, the thoracic duct was cannulated in the cervical region and lymph was collected also from the capsular and hilar lymphatics of the kidney. Blood samples were drawn from the carotid artery.

Immunelectrophoretic analysis of blood plasma and lymph was performed according to the micromethod of

¹ I. RUSZNYÁK, M. FÖLDI, and GY. SZABÓ, *Lymphatics and Lymph Circulation* (Pergamon Press, Oxford-London-New York-Paris 1960).

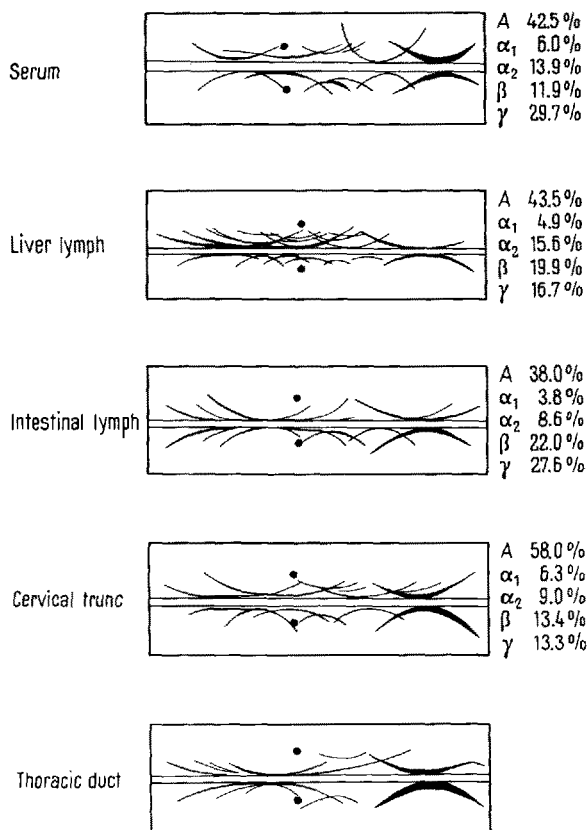


Fig. 1. Immunelectrophoretic and paper electrophoretic analysis of dog serum and lymph.

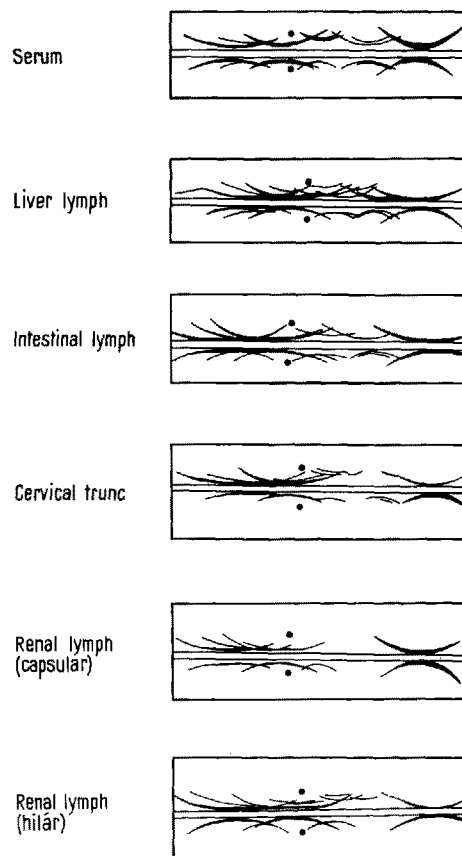


Fig. 2. Immunelectrophoretic pattern of blood serum and lymph samples of different origin of the same animal.