reines Wasser. Bei absteigender Chromatographie (im Dunkeln) wandert die Lösungsmittelfront in 120 min um ca. 20 cm. Die Rf-Werte einiger Vitamine betragen unter diesen Bedingungen:

Thiamin 0; Pyridoxin 0,04; Riboflavin 0,12; Cyanocobalamin 0,14; Nicotinsäureamid 0,18; *p*-Aminobenzoesäure 0,70; Folsäure 0,74; Panthenol 0,85; Na-riboflavinphosphat 0,88; Calcium-pantothenat 0,96.

Summary. A simple new method is described for the separation of water-soluble vitamins, using ion exchange-papers.

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The Effect of Sodium Nitrite on Red Cell GSH[1]

Introduction. In 1946 KEILIN and HARTREE[2] reported that nitrite resulted in the oxidation of red cell glutathione (GSH). It has been generally accepted that this occurs, and similar observations have formed the foundation of several other studies of red cell GSH[3,4]. The investigations reported briefly in this communication demonstrate that nitrite does not oxidize glutathione intracellularly, but that the apparent disappearance of GSH after incubation with nitrite is probably due to *in vitro* interaction of nitrous acid with GSH during the procedure for determining GSH.

Methods. These studies were carried out using human red cells obtained from a polycythemic donor. GSH determinations were carried out by a modification of the method of ELLMAN[5]: Proteins were precipitated with metaphosphoric acid (MPA) and salt and the filtrate added to a cuvette containing 0.5 *M* phosphate buffer pH 7.5. Color was then developed by the addition of 5, 5'-dithiobis (2-nitrobenzoic acid).

Results. It first became apparent to us that nitrite does not actually result in intracellular oxidation of GSH when we observed that repeatedly washing red cells in physiologic salt solution (without glucose) resulted in return of the GSH level to a concentration equal to that before treatment with nitrite. It is evident from Table I that this effect depends upon the number of washings and the volume of washing solutions. Identical results were obtained when the nitroprusside method[6] for determining GSH was employed. It is undoubtedly because of the large number of washings required that other investigators have not noticed this effect.

Tab. I. GSH measured in RBC after nitrite treatment and saline washing (% of initial value)

	Volume of washing solution 1–2	6	10
Washings	%	%	%
1	5	0.5	5
2	0.5	7	—
3	5	53	86
4	1	82	—
5	—	—	110
6	—	—	—
7	—	—	97

When nitrite was mixed with GSH and the analytic procedure, including the addition of metaphosphoric acid (MPA), was carried out in the usual manner, virtually all of the GSH had disappeared. However, if nitrite was added to the cuvette containing GSH and buffer, there was no loss of GSH and no interference with the GSH determination. The experiment depicted in Table II was therefore carried out. A solution approximately 25 mg of GSH per 100 ml of 0.5 *M* phosphate buffer, pH 7.0 was mixed with an equal volume of freshly prepared solutions of 0.25%, 0.12%, 0.06%, 0.03%, and 0.015% sodium nitrite. A control aliquot of GSH was incubated with physiological saline. Two-tenths of a milliliter of each nitrite-GSH mixture was then added to each of two tubes containing 1.8 ml of distilled water. To one of these tubes 3 ml of NaCl saturated 1.67% MPA was added, as is done in our modification of the Ellman technique for GSH determination. To the duplicate tube 3 ml of 0.5 *M* phosphate buffer, pH 7.5 was added instead of MPA. After standing for 5 min, GSH determinations were carried out on each of the mixtures in such a way that the final contents of each cuvette with respect to salt, MPA, and buffer were identical. As indicated in Table II, it was found that when a mixture of nitrite and GSH had been treated with MPA, virtually all of the GSH had been destroyed. In contrast, when phosphate buffer, pH 7.5 had been substituted for MPA the GSH concentration was unaffected by the presence of nitrite.

Preliminary studies have suggested that only slightly more than 1 μM of nitrous acid was required to destroy 1 μM of GSH. It is probable that the products of the reaction are NH_2OH and NH_3[7].

Discussion. These studies demonstrate that the apparent destruction of red cell GSH by sodium nitrite is an artifact produced during the protein precipitation step in the determination of GSH. The results emphasize the difficulty in removing nitrite from red cells, a phenomenon which we have observed previously in studies involving methaemoglobin reduction. The fact that high concentrations of methaemoglobin can exist in erythrocytes with-

Tab. II. GSH remaining after incubation with nitrite

	Mg% sodium nitrite in incubation mixture 120	60	30	15	$7^1/_2$	0
Incubated in acid	1.2	2.3	0.5	0.2	0	100
Incubated in buffer	115.4	98.2	98.5	94.5	95.4	100

[1] Supported in part by U.S.P.H.S. Grants H-5313 and H-5292.

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out appreciable effect on intracellular GSH, indicates that GSH-mediated reduction of methaemoglobin[8], 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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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 plasma[3] and human serum[4], 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 ($F = 2.05$; $p \geqq 0.05$), 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 values[2].

Among the 250 samples tested, 26 sera which did not show hemolysis[2] were found to have enzyme activities which were significantly elevated. The established ubiquity of polyoma virus in mouse colonies[7] and recent studies which have demonstrated an increase in LDH activity following the inoculation of mice with filtrates of a variety of murine tumors[8] 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

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Normal values for serum LDH in 5 inbred strains of mice

Strain	Number of mice	Age (months) 1–3 Range	Mean	4–6 Range	Mean	7–9 Range	Mean	10–12 Range	Mean	13+ 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