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Publication Date
2023-09-06

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LBL-1367

DECREASED SURFACE CHARGE AND INCREASED DESTRUCTION OF RBC FOLLOWING PARENTERAL ADMINISTRATION OF NEURAMINIDASE

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This work was supported, in part, by a Public Health Service Research Career Development Award (5-K4-HL-17693-03) from the National Heart and Lung Institute (Dr. Landaw), by Postdoctoral Fellowships from the Bay Area Heart Research Committee (Dr. Tenforde, 1969-71) and National Cancer Institute (1-F2-CA-52469) (Dr. Tenforde, 1971-72), and by Cancer Research Funds from the University of California.

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ABSTRACT

Female LAF_1 mice were given single or repeated injections of *V. cholerae* neuraminidase, and the effects on circulating RBC surface charge and lifespan were determined. As had been noted previously for RBC incubated *in vitro* with neuraminidase, intravenous injection of this material caused a rapid decrease in RBC surface charge of approximately 14 percent. In addition, there was destruction of approximately 3 percent of young or old RBC cohorts within 24 hours of a single intravenous injection of active neuraminidase. The surviving RBC in these cohorts showed accelerated senescence, with the mean potential lifespan shortened by 17 percent.

Mice given repeated doses of active neuraminidase intravenously showed a decreased hematocrit and marked splenomegaly, along with lowered reticulocyte percentage. Such results are consistent with the above-noted RBC destruction, as well as with the previously demonstrated inhibition of endogenous erythropoietin, rendering the bone marrow incapable of responding to the ensuing anemia. It therefore appears that intravenously administered neuraminidase mimics some of the kinetic disturbances seen in the anemia of chronic disease.

INTRODUCTION

The anemia of chronic disorders (infection, inflammation, and malignancy) has been of interest to investigators for some time. Reversal of this anemia observed after tumor resection, or successful eradication of an infectious process[1,2] suggests that a toxic material may be responsible for the observed alterations in erythropoiesis. It has recently been demonstrated that bacterial neuraminidase injected intravenously is capable of inhibiting the biological activity of erythropoietin[3]. Neuraminidase may therefore be the trophic hormone inhibitor proposed earlier by Cartwright[2] to exist during states of chronic infection and malignancy, since an increase in the level of available neuraminidase could limit the ability of the bone marrow to respond to an anemic challenge[3]. Another alteration characteristic of this anemia, namely decreased erythrocyte (RBC) survival[4,5], may result from modification _in vivo_ of glycoproteins at the RBC surface by neuraminidase, similar to that seen _in vitro_[6,7]. To test this latter hypothesis, studies were made of RBC surface charge and survival in mice, following intravenous injection of bacterial neuraminidase.

MATERIALS AND METHODS

Animal studies were performed in female LAF_1/J mice approximately 2-3 months of age (Jackson Laboratory, Bar Harbor, Maine). Except where indicated, mice were injected intravenously with 100 units of _V. cholerae_ neuraminidase (Behring Diagnostics, Woodbury, New York) without anesthesia. Neuraminidase from this bacterial source is free of phospholipase C, aldolase, and protease contaminants. Erythrocytes incubated _in vitro_ with this material do not show the immediate sphering and lysis observed with neuraminidase extracts from _Clostridial_

sources[8]. Control mice were injected intravenously either with neuraminidase inactivated by 10-60 minutes in a boiling water bath, or with the buffer diluent present in the commercially supplied enzyme preparation.

Erythrocyte surface charge was measured by the technique of microelectrophoresis. At times ranging from 3 hours to 93 days following intravenous injection of 100 units of neuraminidase (N'ase), blood was collected by cardiac puncture into heparinized syringes and stored at 4°C. Immediately prior to study, blood samples were diluted with 400 volumes of a solution consisting of 90 parts of saline and 10 parts of 0.067 M Sorensen phosphate buffer (pH 7.0 $\pm$ 0.1, ionic strength 0.151, temperature 25°C). This solution is referred to hereafter as saline/phosphate buffer. Electrophoretic measurements were completed within 3 hours after drawing the blood samples.

Treatment of RBC with N'ase _in vitro_ was performed with blood obtained by cardiac puncture. The RBC were washed 3 times in saline/phosphate buffer (centrifugation at 1100 g, 5 minutes), and 1 volume of packed RBC was mixed with 2 volumes of N'ase solution (500 μ/ml) and incubated at 37°C for 60 minutes. The RBC were then washed twice with saline/phosphate buffer. Control RBC were treated in an identical manner using heat-inactivated N'ase.

Microelectrophoretic studies were carried out with a Zeiss Cytopherometer (Carl Zeiss, New York) using a $Zn/ZnSO_4$ electrode assembly[9], at a temperature of 25 $\pm$ 0.1°C, and an electric field strength of 3.3 volts/cm. The mobilities of 20 RBC were recorded at each of the 2 stationary levels within the microelectrophoresis chamber. Measurement of the electrophoretic velocity of each cell was made with the

polarity of the applied field in forward and reverse directions. The 2 measurements were then averaged in order to cancel out effects due to mechanical fluid drift within the chamber.

The zeta potential (ζ) was derived from the directly measured electrophoretic mobility (μ) through the Smoluchowski relation:

$$\zeta = \frac{4\pi\eta\mu}{D} \qquad (1)$$

In equation (1) (η) and (D) are, respectively, the viscosity and dielectric constant of the suspending medium. The surface charge density (σ) was then computed from (ζ) by means of the Couy-Chapman equation:

$$\sigma = \frac{10^3 DRT}{2\pi}\left[\sum_i n_i\left(e^{-z_i e\zeta/kT}-1\right)\right]^{1/2} \qquad (2)$$

In equation (2), (e) is the electric charge, (k) is Boltzmann's constant, (R) is the molar gas constant, and (T) is the absolute temperature. The summation extends over all ionic species in the saline/ phosphate buffer medium. Ion concentrations (n_i) are expressed in molar units, and ion valences (z_i) are positive for cations and negative for anions. When all physical constants are expressed in MKS units, (σ) calculated from equation (2) has the units of coulombs/ meter^2. A detailed discussion of the validity of equations (1) and (2) for RBC in physiologic media has been given elsewhere[10].

Red blood cell survival was studied in mice using the method of

endogenous ^{14}CO production following *in vivo* labeling of a cohort of RBC with glycine-2-^{14}C [11]. Neuraminidase was injected intravenously 18 hours, 3-7 days, or 27-32 days following cohort labeling. The effects of N'ase could then be followed for RBC being formed in the bone marrow, newly-emerged circulating RBC, or mature RBC, respectively[12]. The ^{14}CO excretion rates were determined in groups of 5 female mice for at least 5 hours before and 24 hours after injection of 100 units of active or heat-inactivated N'ase per animal, or following injection of buffer diluent. The results are expressed as DPM of ^{14}CO recovered during the 24 hour period immediately following injection of the test material. From the excess ^{14}CO above control values, the fractional destruction of the RBC cohort at risk was also calculated, using the measured incorporation of glycine-2-^{14}C into RBC hemoglobin heme in normal controls, together with an equation relating the size of the cohort versus time after cohort production[11]. In the experiments performed with multiple injections of active or boiled N'ase, the size of the cohort at risk was corrected for any excess ^{14}CO appearing as a result of prior RBC destruction. With this method the destruction of as little as 0.2 to 0.3% of the cohort at risk can be reliably distinguished from background fluctuations in the rate of ^{14}CO excretion.

In a separate experiment, groups of 5 female mice were given 4 consecutive daily injections of active N'ase, boiled N'ase, buffer diluent, or boiled trypsin (protein concentration equal to that in the commercial N'ase preparation); animals were sacrificed on the day following the 4th injection, and splenic weight, hematocrit, and reticulocyte percentage determined using standard techniques.

Osmotic fragility measurements were made on N'ase-treated and control (buffer diluent or boiled N'ase) RBC. Erythrocytes were washed twice with saline (1100 g, 5 minutes), and were incubated in 2 volumes of neuraminidase solution (500 μ/ml) at 37°C for 1 hour. The RBC were then washed twice more with saline. Osmotic fragility was determined by the method of Parpart et al[13].

As a further check on the biologic activity of heat-inactivated N'ase (1 hour in a boiling water bath), 500 units of heat-inactivated N'ase were incubated with 4 IRP units of human urinary erythropoietin for 60 minutes at room temperature. Assay for erythropoietic activity was then determined in female LAF_1/J mice recovering from CO-induced polycythemia.*

RESULTS

A. ERYTHROCYTE SURFACE CHARGE:

Table I summarizes the effects of N'ase on the surface charge and electrophoretic mobility of LAF_1/J RBC *in vitro* and *in vivo*. Surface charge reduction *in vitro* was maximal after approximately 30 minutes of incubation. Following a 1 hour incubation the reduction in the net negative surface charge was 17.2% ($p < 0.001$). This reduction is comparable to that observed by Weiss et al[6,7] for RBC from HACR Swiss mice following *in vitro* N'ase treatment.

A charge reduction of 14.3% ($p < 0.001$) was noted *in vivo* 3 hours after intravenous injection of active N'ase. Statistically significant decreases ($p < 0.001$) were also observed at 24 hours and 10 days post-injection, as compared to controls injected with heat-inactivated N'ase. Thirty or more days after injection, the surface

*Kindly performed by Dr. J. F. Garcia, Donner Laboratory.

charge density was not significantly different from control values ($p > 0.35$). No effect on surface charge was noted following incubation in vitro or injection in vivo of boiled N'ase relative to RBC treated with buffer diluent. In all cases, the RBC populations were uniform with respect to surface charge reduction; no sub-population of unduly sensitive or insensitive RBC was noted, as reflected by the small standard errors reported in Table I.

B. ERYTHROCYTE SURVIVAL:

Table II summarizes the data on immediate RBC destruction over a 24 hour period following intravenous injection of active N'ase, heat-inactivated N'ase, or buffer diluent. After administration of active N'ase, a marked increase in ^{14}CO excretion rate was noted within 5 hours (Figure 1). Over the first 24 hours post-injection, RBC destruction amounted to approximately 3% of the cohort at risk in both young (3-7 days) and old (27-32 days) cohorts. Following injection of heat-inactivated N'ase, no increase in ^{14}CO excretion was seen in a 4 day-old cohort, while approximately 0.7% of a 27 day-old cohort was destroyed by the same treatment. Intravenous injection of saline or the pH 5.5 buffer diluent produced no detectable RBC destruction.

Figure 1 depicts the effect of N'ase on ^{14}CO excretion in 2 groups of mice studied 27 days after glycine-2-^{14}C injection. In one group, 4 injections of active N'ase were given at 20-25 hour intervals. Each injection gave rise to an immediate increase in ^{14}CO excretion, with the maximal excretion rate being reached 5-10 hours post-injection. When the ^{14}CO excretion was stated in terms of fractional destruction of the cohort at risk, 3.2% was destroyed by the

first injection over the subsequent 24 hour period, 4.0% by the 2nd, 4.4% by the 3rd, and 5.2% by the 4th injection. In a second group of mice also depicted in Figure 1, the 1st injection of active N'ase produced a similar increase in ^{14}CO excretion; however an injection of saline 24 hours later failed to produce RBC destruction, and the subsequent ^{14}CO excretion rate declined exponentially towards the normal baseline level. A similar result was noted for a 4 day-old cohort when an injection of active N'ase was followed 24 hours later by an intravenous injection of the buffer diluent.

Table III indicates RBC survival in 3 groups of mice each given 2 injections of active N'ase at 18 hours and 3 days, 4 and 7 days, or 27 and 30 days, respectively, following cohort labeling. A shortening of mean potential lifespan (time of senescent RBC death) from the normal value of 53 days to 44 days was seen ($p < 0.001$), without alteration in the rate of random (age-independent) RBC destruction (Figure 2). The mean overall RBC lifespan, which takes both modes of RBC death into account, was reduced from 46 days in the controls to 40 days in the N'ase-treated mice ($p < 0.025$), or a shortening in lifespan of 17% for those RBC surviving the initial destruction. The total recovery of ^{14}CO from destruction of circulating RBC in the normals (0.345% of injected glycine activity) did not differ significantly from that obtained in N'ase-treated mice (0.364%), confirming that all of the cohort destruction was accounted for by this technique[14]. The validity of the equation used for the determination of cohort size[11] was checked by direct measurement of circulating RBC hemoglobin heme specific activity at 27 days following injection of glycine-2-^{14}C. The predicted cohort size was 0.291% of injected glycine activity, whereas direct measurement of specific activity and

mouse blood volume yielded a value of 0.301%.

In order to rule out the possibility that some of the ^{14}CO may have arisen from non-hemoglobin heme sources, and to determine the effect of N'ase on the maturing RBC precursors in the sites of RBC production, N'ase was given to a group of 5 mice 15 hours following injection of labeled glycine. As shown in Figure 3, no significant increase in ^{14}CO excretion was seen in the experimental group, as compared to the uninjected controls.

C. MISCELLANEOUS STUDIES:

Osmotic fragility curves were identical for RBC incubated *in vitro* with active or boiled N'ase, or saline; 50% hemolysis occurred in 0.56 to 0.60% NaCl in all 3 groups.

Splenic weight, hematocrit, and reticulocyte percentage were not significantly altered in mice receiving 4 daily doses of buffer diluent or boiled trypsin solution (Table IV). Following active N'ase injections, splenic weight increased more than 2 1/2 times normal, with a statistically significant decrease in hematocrit from 54% to 42% ($p < 0.001$), and a slight *decrease* in reticulocyte percentage from 2.7% to 1.9% ($p > 0.05$). Following heat-inactivated N'ase, splenic weight averaged more than twice normal, with a statistically significant decrease in hematocrit and a slight *increase* in reticulocyte percentage.

To test for the possibility that heat-inactivated N'ase contains residual anti-erythropoietin activity, 4 IRP units of human urinary erythropoietin were incubated with 500 units of heat-inactivated (60 minutes in a boiling water bath) N'ase for 60 minutes at room temperature. Each mouse assay animal was injected with approximately 0.4 IRP units of erythropoietin or with an equivalent amount of erythro-

poietin incubated with the heat-inactivated N'ase. Forty-eight hours later, ^{59}Fe was given intravenously, and incorporation into circulating RBC was measured 72 hours later. Results showed an ^{59}Fe incorporation of 20.7 $\pm$ 1.6 (SE)% of the injected dose in control mice, and 20.4 $\pm$ 1.2% in mice receiving erythropoietin incubated with heat-inactivated N'ase. Previous studies[3] had shown that, in this system, 5 units of active N'ase are sufficient to reduce the biologic effect of 4 IRP units of erythropoietin, indicating that the boiled material was more than 99% inactivated.

DISCUSSION

In the present experiments, it was observed that approximately 3% of young and old RBC cohorts were destroyed within 24 hours after injection of N'ase. Further, when the long-term fate of these cohorts was followed, overall survival of the remaining RBC was decreased by 17%, due to a shortening of the time of senescent death. Neuraminidase did not appear to cause intramedullary destruction of developing RBC precursors, nor was any increase in turnover of non-hemoglobin heme proteins seen in these experiments. Our results also indicate that intravenously administered V. cholerae neuraminidase causes a decrease in mouse RBC surface charge. The extent of alteration in vivo (-14%) was comparable to the maximum achievable in vitro (-17%)[6,7]. No age-related susceptibility could be detected, since charge reduction was uniform in the populations studied.

The joint occurrence of altered RBC surface charge and immediate RBC destruction is consistent with recent theories concerning RBC destruction by cells of the reticuloendothelial system. A dominant factor in this process appears to be the recognition of RBC with al-

tered membrane properties[15,16]. It has been suggested by Pollack et al[17] that the net negative charge at the RBC surface tends to separate RBC and prevent clumping. If such clumps were to form in the spleen, the RBC would be susceptible to trapping and destruction in this organ. Similarly, altered surface charge might be detected directly by phagocytic cells[18], or new and/or altered antigenic sites may be exposed subsequent to removal of sialic acid residues [19-22]. That gross alterations in the RBC membrane were not induced by N'ase treatment is suggested by the normal osmotic fragility of these cells. The normal surface charge noted 30 days following N'ase injection, at a time when approximately one-half of the cells initially altered should still have been in the circulation, suggests that some of the N'ase-induced damage is repairable. It seems likely that this repair was performed at a cost of increased turnover of intracellular or membrane enzymes, which cannot be replaced by the mature RBC[23]. Since the process of senescence may be linked ultimately to the depletion of RBC enzyme systems, the natural consequence of such repair would be an accelerated senescence[24].

Mice given repeated injections of N'ase exhibited both a decrease in hematocrit and reticulocyte percentage. This observation is consistent with immediate RBC destruction noted in the cohort experiments as well as with recent findings of Schooley and Mahlmann concerning inactivation of erythropoietin *in vivo* by N'ase[3]. Since inactivation of erythropoietin *in vivo* can completely suppress RBC production in the mouse[25], animals thus treated would be unable to respond to the resultant RBC destruction with increased production of new RBC. A distinctly different pattern was found following injection of heat-inactivated N'ase, which was shown not to inactivate

erythropoietin[3], even when present in great excess. After administration of inactive N'ase, a slight decrease in hematocrit was appropriately associated with an increase in reticulocyte percentage.

The mechanism by which a small amount of RBC destruction is produced through injection of heat-inactivated N'ase is not clear from these experiments, although it is not due to an alteration in surface charge. Boiled N'ase was without effect on a young (4 day) cohort; however, 0.7% of an older (27 day) cohort was destroyed by such treatment. When the same dose of boiled N'ase was given to an old cohort 48 hours after injection of active N'ase, approximately 3.0% of the cohort at risk was destroyed. This synergism suggests that a potentially cytotoxic contaminant may exist in the enzyme preparation, or may be produced upon heating the active material. This contaminant can, however, account for only about 15% of the initial RBC destruction caused by a single injection of active N'ase, and for only 50% of the increase in splenic size resulting from the repeated injections. The splenomegaly which occurs after injection of N'ase probably does not result from injection of foreign protein _per se_, since administration of similar amounts of heat-inactivated trypsin, a protein of comparable molecular weight, failed to produce an increase in splenic size. Whether the splenomegaly is a result of increased RBC destruction or is a tissue response to the N'ase preparation is not clear from these studies, but reticuloendothelial hyperplasia has been commonly noted in tumor-bearing animals and man[22], and can be considered as another factor leading to reduction in RBC lifespan.

Neuraminidase activity has been shown to be present in tumor cells[26,27] and elevated in tumor-bearing animals[28], and it has been suggested that this enzyme may be elaborated as a host defense

against increased levels of mucoproteins resulting from destruction of tumor as well as tissue ground substance[29,30]. Of interest in this regard is the occasional occurrence of frank hemolytic anemia accompanying mucin-producing adenocarcinoma of the gastrointestinal tract[22] or ovary[31], and in infections with *V. cholerae*[32], the commercial source for N'ase in the present experiments. In preliminary experiments (J. C. Schooley, unpublished observations), the serum of tumor-bearing LAF_1 mice has been shown to inhibit erythropoietin both *in vivo* and *in vitro*. Experiments are now in progress to further characterize this important finding.

Whether other molecules which can be inactivated by N'ase *in vitro* such as follicle-stimulating hormone (FSH)[33], leuteinizing hormone (LH)[34], and transferrin[35], are inactivated *in vivo* under similar circumstances is not known. Such inactivation could account in part for the endocrine and metabolic disturbances that generally accompany chronic disease states. In view of the large number of hormones and membrane surfaces which can be altered by the action of neuraminidase, we propose that further *in vivo* studies of this enzyme system should prove quite valuable in gaining a deeper understanding of host reactions in chronic infection, inflammation, and malignancy.

ACKNOWLEDGEMENTS

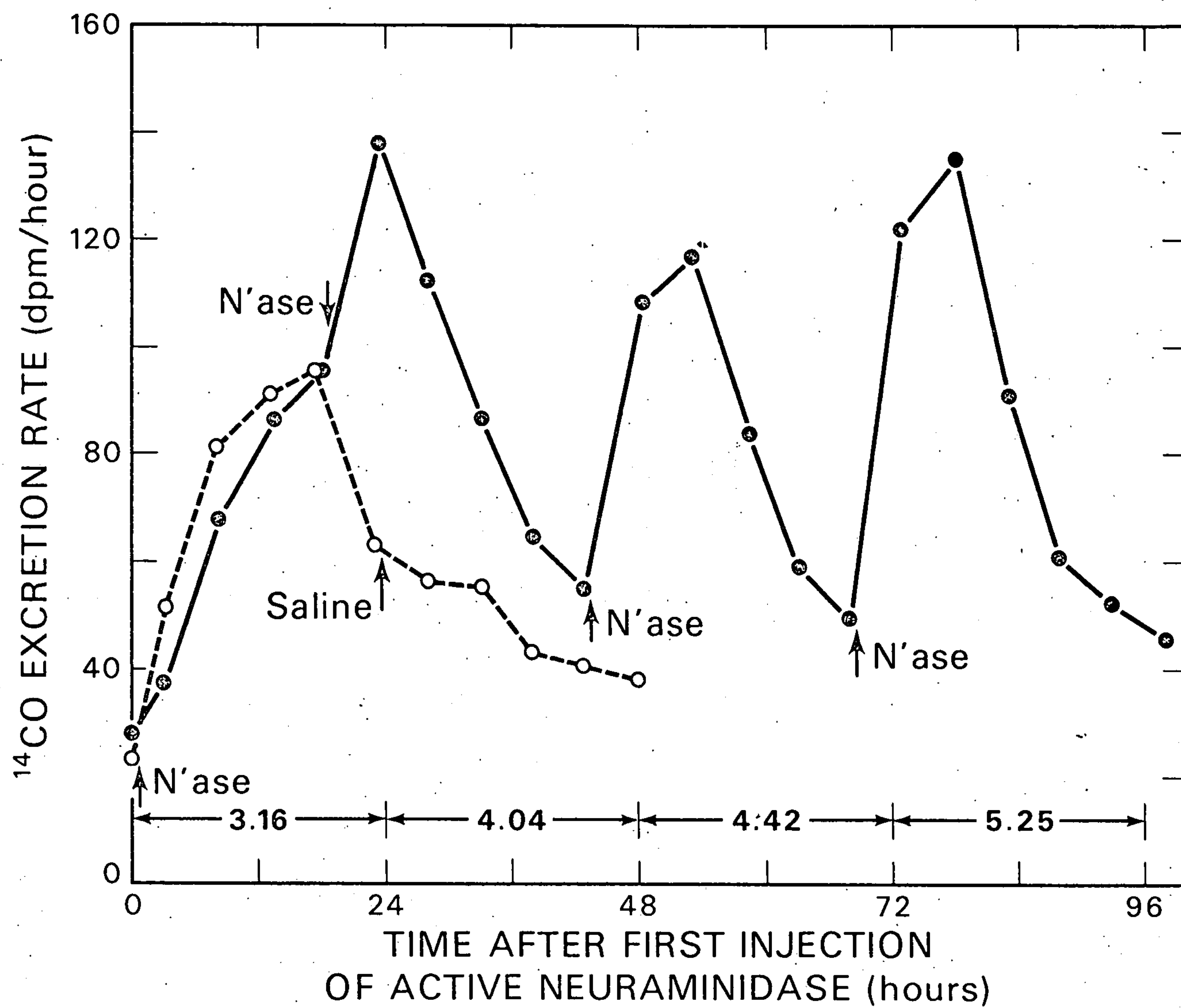
The dedicated technical assistance of Sandra Kay Bristol and secretarial assistance of Linda McColgan are gratefully acknowledged.

FIGURE LEGENDS

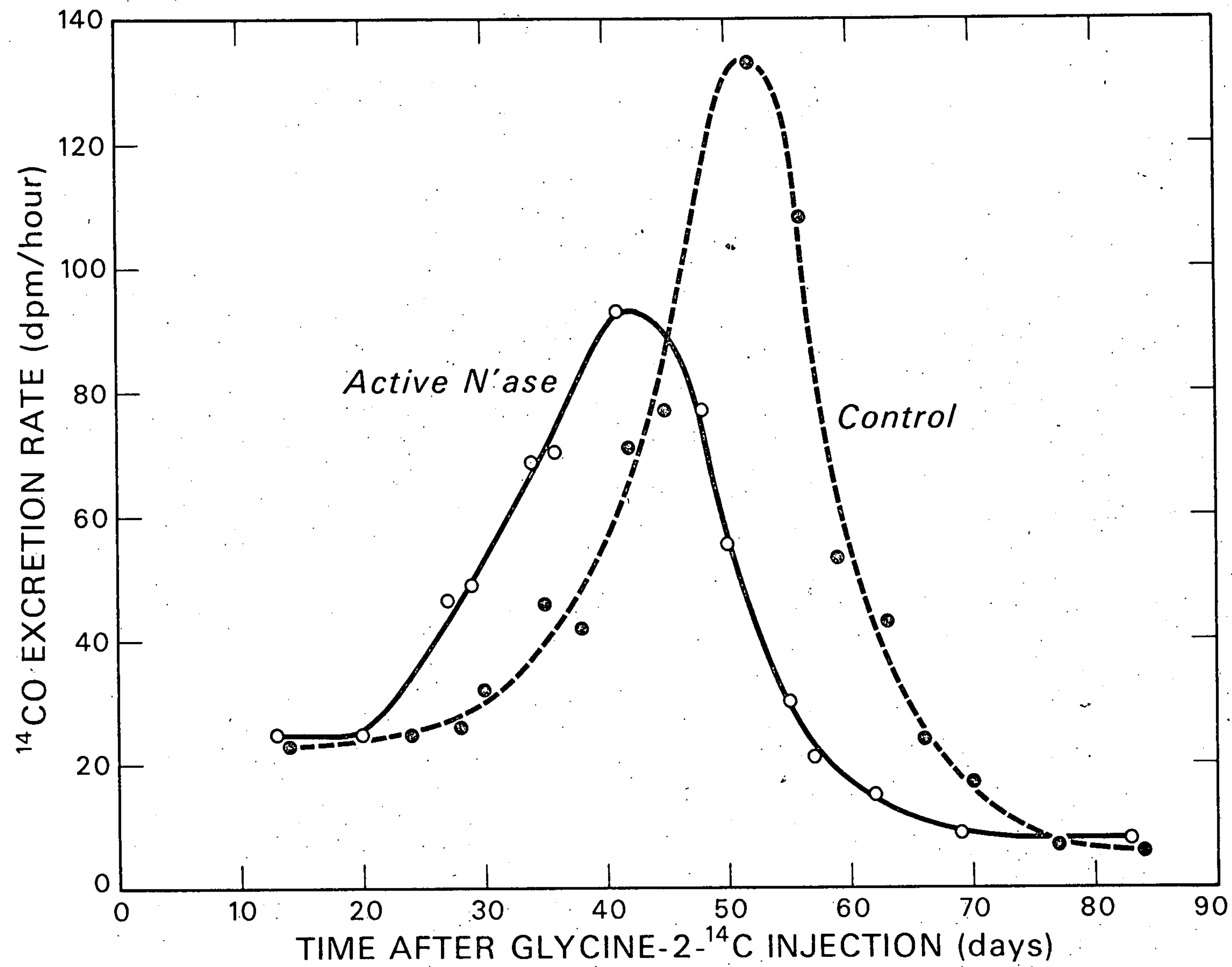
Figure 1: Immediate RBC destruction produced by intravenous injection of active neuraminidase (N'ase). In one group of mice (closed circles, solid line) N'ase was injected as shown (arrows), resulting in increased ^{14}CO excretion from a 27 day-old cohort. The percent of the cohort at risk destroyed by each injection is shown by the number below each 24-hour time period. In a second group of mice (open circles, dashed line), the first injection of N'ase was followed by an injection of saline, resulting in no further increase in RBC destruction.

Figure 2: Long-term ^{14}CO excretion in a group of normal mice (solid circles, dashed line) and a group of mice given injections of active N'ase 4 and 7 days after injection of labeled glycine (open circles, solid line). The time of maximal RBC destruction as the result of senescence was reduced from 53 days in the controls to 42 days in the N'ase-treated group.

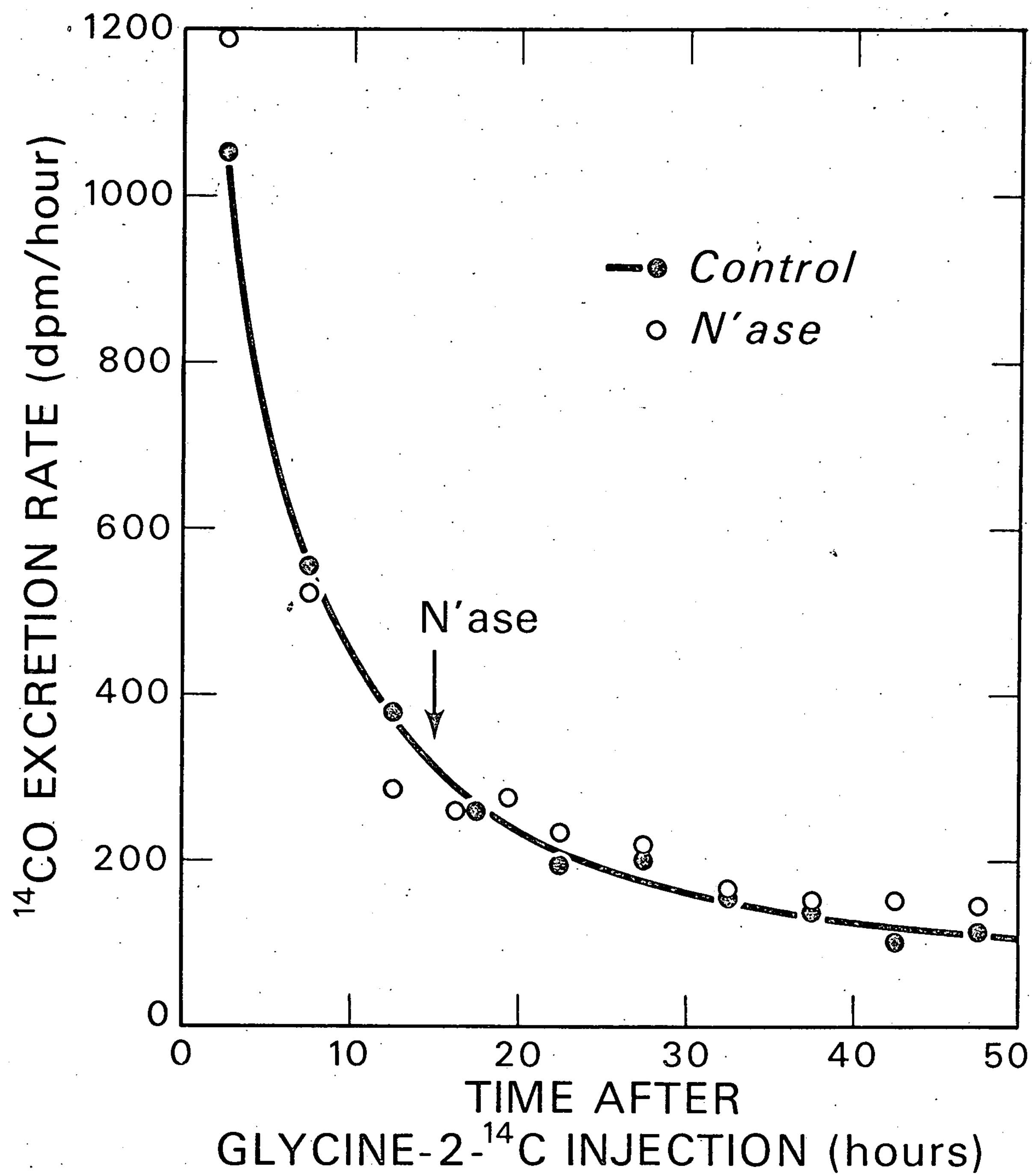
Figure 3: "Early labeled peak" for ^{14}CO production in normal mice (closed circles, solid line), and in mice injected with active N'ase at 15 hours after injection of labeled glycine (open circles). No effect of N'ase on subsequent heme turnover can be detected at this time.



160
120
80
40
0
^{14}CO EXCRETION RATE (dpm/hour)
N'ase
Saline
N'ase
N'ase
N'ase
3.16
4.04
4:42
5.25
0
24
48
72
96
TIME AFTER FIRST INJECTION
OF ACTIVE NEURAMINIDASE (hours)



140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
14CO EXCRETION RATE (dpm/hour)
TIME AFTER GLYCINE-2-14C INJECTION (days)
Active N'ase
Control



1200
1000
800
600
400
200
0
0
10
20
30
40
50
Control
N'ase
N'ase
^{14}CO EXCRETION RATE (dpm/hour)
TIME AFTER
GLYCINE-2-^{14}C INJECTION (hours)

TREATMENT		ELECTROPHORETIC MOBILITY (μ/second/volt/cm) CONTROL	NEURAMINIDASE	NET NEGATIVE SURFACE CHARGE DENSITY (coulombs/meter²) CONTROL	NEURAMINIDASE	ALTERATION IN RBC SURFACE CHARGE (%)	"p" VALUE
IN VITRO	1 hour*	1.31 ± 0.03#	1.09 ± 0.02	1.53×10^{-2}	1.27×10^{-2}	- 17.2	< 0.001
IN VIVO	3 hours**	1.37 ± 0.01	1.18 ± 0.01	1.60	1.37	- 14.3	< 0.001
	1 day	1.21 ± 0.02	1.07 ± 0.01	1.41	1.24	- 11.7	< 0.001
	10 days	1.23 ± 0.02	1.08 ± 0.01	1.44	1.26	- 12.6	< 0.001
	30 days	1.18 ± 0.02	1.16 ± 0.01	1.37	1.35	- 1.7	N.S.
	76 days	1.30 ± 0.01	1.29 ± 0.02	1.52	1.51	- 0.8	N.S.
	93 days	1.24 ± 0.01	1.26 ± 0.01	1.45	1.47	+ 1.5	N.S.

#Mean ± S.E.

*1000 units of heat-inactivated (control) or active N'ase per ml of RBC

**100 units of heat-inactivated (control) or active N'ase per animal

TABLE I

EFFECT OF NEURAMINIDASE IN VITRO AND IN VIVO ON ELECTROPHORETIC MOBILITY AND SURFACE CHARGE OF MOUSE ERYTHROCYTES

TREATMENT	NUMBER OF GROUPS	^{14}CO EXCRETION (DPM IN 24 HOURS)	"p" VALUE	FRACTION OF RBC COHORT DESTROYED (%)
		YOUNG RBC COHORT (3-7 DAYS)		
NONE	4	1376 ± 32*	--	--
BUFFER DILUENT	1	1318	N.S.	--
BOILED NEURAMINIDASE	1	1373	N.S.	--
ACTIVE NEURAMINIDASE	2	2908 ± 26	< 0.001	3.42
		OLD RBC COHORT (27-32 DAYS)		
NONE	8	506 ± 12	--	--
BOILED NEURAMINIDASE	1	763	< 0.001	0.67
ACTIVE NEURAMINIDASE	2	1764 ± 74	< 0.001	3.30

*Mean ± S.E. for groups of 5 mice each, normalized for injection of 10 μCi of glycine-2-^{14}C per mouse.

TABLE II

EFFECT OF A SINGLE INTRAVENOUS INJECTION OF ACTIVE NEURAMINIDASE (100 units/mouse) ON RBC DESTRUCTION IN FEMALE MICE

PARAMETER	CONTROLS	NEURAMINIDASE-TREATED	"p" VALUE
RANDOM HEMOLYSIS (%/day)	0.61 ± 0.12*	0.47 ± 0.10#	N.S.
MEAN POTENTIAL LIFESPAN (days)	52.8 ± 0.3	44.1 ± 1.0	< 0.001
STANDARD DEVIATION ABOUT MPLS (days)	8.8 ± 0.9	7.9 ± 1.8	N.S.
GLYCINE INCORPORATION INTO RBC HGB HEME (%)	0.345 ± 0.022	0.364 ± 0.020	N.S.
MEAN OVERALL RBC LIFESPAN (days)	45.5 ± 1.2	39.9 ± 1.2	< 0.025

*Mean ± S.E. for 4 groups of 5 female LAF_1 mice each

#Mean ± S.E. for 3 groups of 5 female LAF_1 mice each. See text for details of time of neuraminidase injections.

TABLE III

RBC LIFESPAN IN FEMALE MICE GIVEN TWO INTRAVENOUS

INJECTIONS OF ACTIVE NEURAMINIDASE

TREATMENT	SPLENIC SIZE (% BODY WEIGHT)	HEMATOCRIT (%)	RETICULOCYTES (%)
BUFFER DILUENT	0.345 ± 0.009*	53.7 ± 0.5	2.7 ± 0.2
BOILED TRYPSIN	0.321 ± 0.008	51.4 ± 0.6**	2.6 ± 0.2
BOILED NEURAMINIDASE	0.657 ± 0.038#	49.8 ± 0.4#	3.8 ± 0.8
ACTIVE NEURAMINIDASE	0.917 ± 0.039#	41.9 ± 0.6#	1.9 ± 0.7

*Mean ± S.E. for groups of 5 animals each (injected days 1-4; studied day 5)

**$p < 0.02$

#$p < 0.001$

TABLE IV

EFFECT OF REPEATED INTRAVENOUS INJECTIONS OF NEURAMINIDASE ON HEMATOLOGIC PARAMETERS IN FEMALE MICE

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