TIMING OF INTRACRANIAL BLEEDING IN NEWBORN INFANTS

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Red blood cells labeled with the stable tracer ^{50}Cr have been used to investigate the timing of intracranial bleeding in immature newborn infants who required intensive nursery care. The median age at tagging the infant's red blood cells was 7.5 hr after birth. Of 69 infants from whom intraventricular clot samples were obtained at autopsy, 45 infants had the hemorrhage after tagging. In 18 infants part of the hemorrhage occurred before tagging and part afterwards. In six infants the hemorrhage occurred before tagging. For the 31 infants on whom a more precise estimate of the hemorrhage time was available, the data indicated that the intracranial bleeding occurred near the time of death. It is concluded that most of these infants' intracranial bleeding occurred after birth and after their disease process was clearly established.

The use of the stable isotope ^{50}Cr for the tagging of donor red cells has been found to be a safe and practical method. The application of stable tracers, neutron activation analysis, and high-resolution gamma-ray spectroscopy has provided a tool for clinical investigations on high radiation risk subjects such as newborn infants without the administration of radioisotopes.

The use of stable tracers is a desirable alternative to radioactive tracers for clinical investigations in newborn infants and pregnant women. The stable tracer ^{50}Cr has been shown to be as adequate a red cell tag as the commonly used radioactive red blood cell tag, ^{51}Cr (*1–3*). In this paper, the results of a clinical investigation on newborn premature infants using the stable red blood cell tag ^{50}Cr are reported.

Intracranial hemorrhage has been known to occur commonly in very immature infants. We have investigated the time at which intracranial hemorrhage occurs to see if this is before a time when preventive therapy could have been initiated. Newborn premature infants, both those with respiratory distress and those of very low birth weight, in an intensive care nursery were transfused with red cells tagged with ^{50}Cr as early after birth as possible, usually before 12 hr old. If the infant died and an intracranial hemorrhage was found at autopsy, samples of the clot were obtained. The amounts of ^{50}Cr and natural iron in the infant's blood and clot samples were quantitated in vitro by neutron activation analysis.

METHODS AND MATERIALS

Infants with clinical hyaline membrane disease, perinatal asphyxia, or very low birth weight ($<$1,250 gm) often die and at autopsy are found to have intraventricular hemorrhages. These high-risk infants were transfused with donor whole blood in which the red blood cells had been labeled with the stable tracer, ^{50}Cr, following similar procedures used with radioactive ^{51}Cr-labeling for red cell volume and survival studies (*4*). Chromium in the form of sodium chromate enriched to 95.9% ^{50}Cr was used in these studies. About ½ hr after transfusion of the labeled red blood cells, a 1-ml sample of whole blood was taken from the infant. If the infant died, a sample of postmortem blood was taken. If at autopsy intracranial or other clots were found, then samples from these clots were obtained and assayed for ^{50}Cr content.

If a clot contained a significant portion of the tagged red cells, we inferred that a significant portion of the bleed occurred after injection of the ^{50}Cr-tagged blood. The time of hemorrhage, either before or after injection of the ^{50}Cr-labeled blood, was

Received Apr. 17, 1973; original accepted June 20, 1973.

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estimated by determining the amount of ^{50}Cr per total number of red blood cells in the intracranial clot and post-tagging blood samples. The Cr/Fe index, defined as the ^{51}Cr activity in the sample divided by the ^{59}Fe activity in the sample, is the basis for the estimation of time of clot formation. The ^{51}Cr activity arises from activation of the ^{50}Cr, while the ^{59}Fe activity arises from activation of the natural iron in the red blood cells. The blood and clot samples were irradiated at the Georgia Institute of Technology Research Reactor for a period of about 100 hr. A decay period of 200–300 hr after the end of irradiation was used to minimize interference from the 15-hr half-life ^{24}Na.

Some of the problems associated with neutron activation analysis of biological samples have been avoided by counting the induced ^{51}Cr and ^{59}Fe activities with a lithium-drifted germanium [Ge(Li)] gamma-ray detection system. With the superior gamma-ray energy resolution of a Ge(Li) detector, the quantitation of the pertinent activities in the irradiated blood and clot samples was possible without the necessity of chemical separation procedures. Figure 1 graphically shows the advantage of the Ge(Li) detector for the ^{50}Cr determination. This graph is a plot of the gamma-ray energy spectra in the region of the 320-keV peak of ^{51}Cr taken with both Ge(Li) and NaI(Tl) gamma-ray detection systems. Both spectra are from the same sample measured for the same count time of 10 min. The NaI(Tl) data are the open circles with the events per energy interval indicated on the left, while the Ge(Li) data are the dots with the y-axis noted on the right. The NaI(Tl) data indicate only a vague hump for the gamma-ray peak of ^{51}Cr while the Ge(Li) gamma-ray peak is sharp and well defined. Figure 2 shows the advantage of the Ge(Li) detector for the iron determination. This graph presents the gamma-ray energy spectra from an activated blood sample in the energy region around the two ^{59}Fe transitions of 1,099 and 1,292 keV. The open circles are data from a NaI(Tl) detector while the dots are from a Ge(Li) detector. The NaI(Tl) spectral data show only two broad peaks in the region of the two transitions from ^{59}Fe, while the Ge(Li) spectral data show not only peaks from ^{59}Fe but also those from ^{86}Rb, ^{65}Zn, ^{46}Sc, ^{60}Co, and ^{22}Na. The superior gamma-ray energy resolution of a Ge(Li) detector often makes it possible to do neutron activation analysis of biological samples without radiochemical separations.

The Cr/Fe index was determined for the infant's blood sample taken soon after tagging, the postmortem blood sample, and the clot sample. The minimum percentage of the clot formed after tagging is the ratio of the clot index divided by the first blood index. This would be the least portion of the bleed after tagging because of dilution of the ^{50}Cr label within the infant's blood due to blood replacement during clinical management. As an example for a typical infant, the intraventricular clot index was 3.01 and the first blood index was 3.29. These values yield a minimum percentage of hemorrhage after tagging of 91%. Therefore, for this infant, at least 91% of the intraventricular hemorrhage occurred after the ^{50}Cr-tagged blood was given, and we presume that probably all of the bleeding occurred after tagging.

Because of the variability and lack of precision in

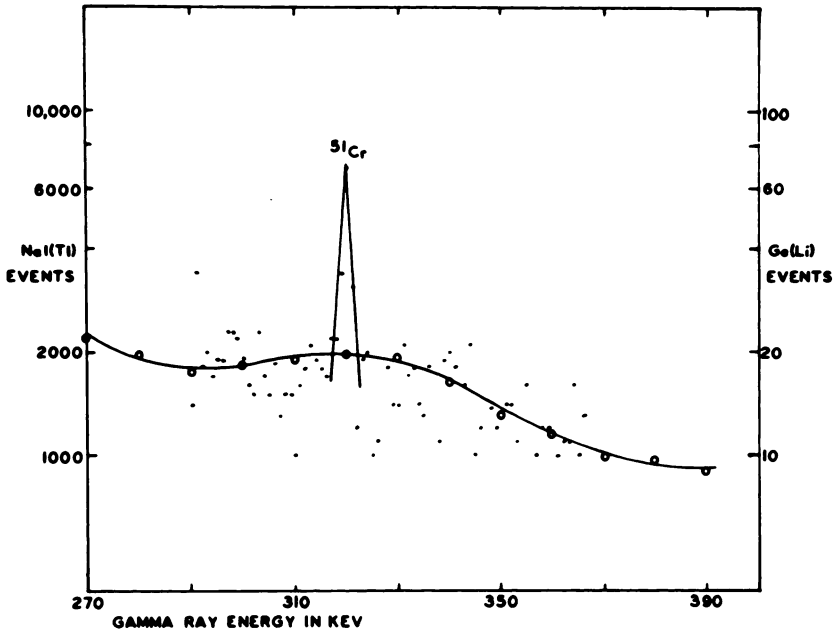

FIG. 1. Gamma-ray spectral data in energy region of ^{51}Cr from neutron-activated blood sample taken with NaI(Tl) detector (open circles) and Ge(Li) detector (dots).

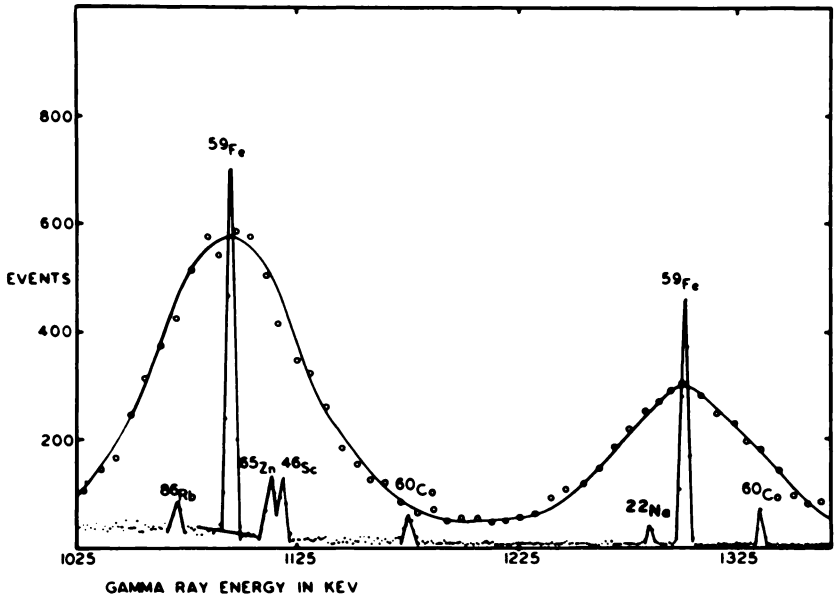

FIG. 2. Gamma-ray spectral data in energy region of ^{59}Fe from neutron-activated blood sample taken with NaI(Tl) detector (open circles) and Ge(Li) detector (dots).

the determination of the minimum percentage of each clot, we have timed the bleeding in three categories. If the minimum percentage for the clot was 70% or greater, it was thought that the hemorrhage occurred primarily after tagging. If the minimum percentage was less than 70% but not less than 30%, then bleeding may have occurred both before and after tagging. If the minimum percentage was less than 30%, then it was believed that hemorrhage occurred before tagging.

To obtain a more precise estimate of the time of intracranial hemorrhage, serial blood samples are now taken from the infants at approximately 24-hr intervals after tagging until they improve clinically or die. By plotting the Cr/Fe index as a function of time after tagging, the time relationship of the ^{50}Cr tag per circulating red blood cell is obtained. The decrease in the index value with time occurs because of the blood sampling and transfusing that occur as part of the infant's clinical management. The Cr/Fe index value determined for the clot sample, when placed on the timing curve determined by the blood samples, provides a means of localizing the time of hemorrhage.

RESULTS

At the initiation of the ^{50}Cr studies, the red blood cell volume for one infant and three adult patients was measured using both ^{51}Cr and ^{50}Cr tracers. These patients required ^{51}Cr blood volumes in their clinical management. Figure 3 presents the correlation between the two measurements for each subject with the ^{50}Cr and ^{51}Cr tags. The values as measured with the two labels were in close agreement.

Subsequently, 276 high-risk newborn infants have received ^{50}Cr-tagged red blood cells. Of these infants, 120 died, and autopsies were performed on 97 infants. Sixty-nine infants were found to have intracranial hemorrhages, and their birth weights ranged from 780 to 3,374 gm with a median of 1,200 gm. For gestational age, the range was from 26 weeks to term with a median of 30 weeks. The time after birth when the infants were tagged ranged from 2.3 to 51.1 hr with a median of 7.5 hr. The time after birth when death occurred ranged from 5.6 to well over 100 hr. The median time of death was 46 hr. The birth weight, gestational age, and age at tagging distributions of all the infants who died were essentially the same and had the same medians as for the infants with clots. However, the age at death distribution appeared to be displaced toward higher age values for all the infants who died, and the median was 55 hr. That is, it appears that the earlier the infant died after birth the more likely was an intracranial clot to be found at autopsy.

The estimated time of the intracranial hemorrhage was determined from the minimum percentage value by the range criteria presented previously. For 45 infants, the majority of the bleed occurred after red cell tagging. There were 18 infants whose minimum percentage of clot formation was between 30 and 70%. For these infants, we estimate that approximately one-half the hemorrhage occurred before tagging and one-half afterwards. Only six infants had the majority of their intracranial bleed before red cell tagging. The ages at tagging for these six infants were 4.9, 5.6, 5.7, 18.1, 36.1, and 40.0 hr. The two infants who were tagged at 36.1 and 40.0 hr old had minimum percentages of 23 and 29%, respec-

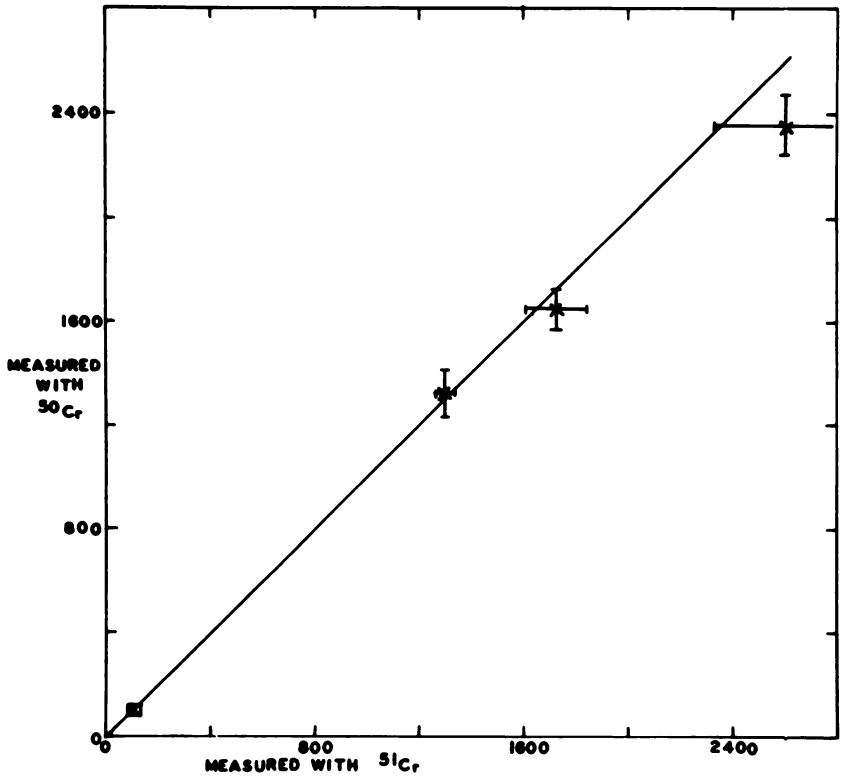

FIG. 3. Comparison of simultaneous measurements of red cell volume with red cell tags ^{50}Cr and ^{51}Cr for four subjects. RBC volumes are shown in milliliters.

tively, so they probably had had their intracranial hemorrhage after birth but before tagging.

For 31 of the 69 infants, sequential blood samples were obtained which permitted a more accurate fix on the time of intracranial hemorrhage. A typical timing curve is shown in Fig. 4. In the example shown, the infant's blood was tagged with ^{50}Cr-labeled red cells 6 hr after birth, and death occurred 48 hr postpartum. The vertical axis represents the Cr/Fe index, and the horizontal axis is the age of the infant in hours. The Cr/Fe index of the intraventricular clot obtained at autopsy was found to be 2.15 ± 0.15. This indicates that the intraventricular hemorrhage probably occurred at 42–44 hr of age or about 5 hr before death of this infant. Table 1 gives the distribution of the times between the estimated occurrence of the intracranial bleed and death for the 31 infants with a timing curve. Fourteen or about one-half the infants investigated appear to have had their intracranial bleed within 12 hr of death.

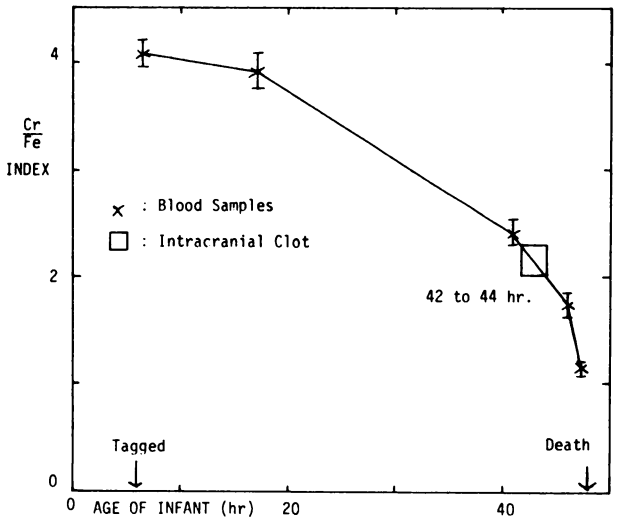

FIG. 4. Typical intracranial clot timing curve determined from Cr/Fe index to time after tagging relationship from five blood samples. Placement of intracranial clot's Cr/Fe index value on curve gives estimated time of hemorrhage as 42–44 hr of age or 5 hr before death.

DISCUSSION

The majority of the infants studied were very premature. Half the infants had a gestational age of 30 weeks or less. From the minimum percentage of clot formed after tagging it appears the large majority of the infants had at least some part of their intracranial hemorrhage after the initial transfusion with tagged red cells. Only 6 out of the 69 infants had their entire bleed before the first transfusion. There did not appear to be any relationship between the primary cause of death and when the major fraction of the bleed occurred.

The results from the timing curves given in Table 1 indicate that the intracranial bleeds for 14 of the 31 infants occurred within 12 hr before death. Only five infants had their intracranial bleed earlier than

TABLE 1. TIME BETWEEN ESTIMATED OCCURRENCE OF INTRACRANIAL BLEED AND DEATH FOR 31 INFANTS

Time between intracranial bleed and death (hr)	Number of infants
0–4	8
5–8	2
9–12	4
13–16	5
17–20	3
21–24	4
25–28	1
>40	4

24 hr before death. The four infants whose intracranial bleed occurred earlier than 40 hr before death, lived for over 100 hr after birth. If these 31 infants are representative of the other 38 infants, then it appears that the intracranial bleed coincides temporally with the terminal event.

One phenomenon which could invalidate the results presented would be the exchange of ^{50}Cr-tagged red blood cells between the clot and circulating blood after the clot was formed. To investigate this possibility, studies were done on two rabbits in which nonlabeled clots were introduced into the lateral ventricle before tagging the rabbit's circulating red blood cells with ^{51}Cr. In both cases, the results showed that there was no uptake in the previously formed clot from the subsequently tagged circulating blood. A reinforcement of this conclusion is given by five infants with known subgaleal clots before tagging. The subgaleal clots had minimum percentages of less than 30% while intraventricular clots in the same infants had minimum percentages of 70% or greater in each case.

The application of stable tracer, neutron activation analysis, and high-resolution gamma-ray spectroscopy has enabled clinical investigations on newborn infants without the radiation exposure risk associated with radioactive tracers. Because of the high-energy resolution capabilities of a Ge(Li) detector, it has been possible to quantitate the particular radioisotopes of interest in neutron-activated blood samples without the problems associated with chemical separation procedures. A reactor, located approximately 250 miles from our hospital, was used for these studies. The use of a stable isotope, ^{50}Cr, for the tagging of donor red cells has been found to be a safe and practical method for the timing of intracranial bleeding in such infants and provides a tool that can be used in most modern hospitals for additional investigations into the cause and prevention of such bleeding.

ACKNOWLEDGMENTS

The assistance of M. E. McLain, Jr., and the Georgia Institute of Technology for the reactor irradiations was greatly appreciated. Appreciation is expressed to H. Sundall, J. Raye, R. Gutberlet, G. Faxelius, S. Swanstrom, G. Dolanski, R. Cotton, J. Relier, and M. Dehan for their assistance in obtaining the blood and clot samples. This work was partially supported by the USAEC through Contract #AT-1-2401, the National Institutes of Health Grant #HL14214, and National Heart Institute Grant #5P01-HE08195.

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