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# A Comparison of [$^{35}$S]Sodium Sulfate and [$^{75}$Se]Selenomethionine as Platelet Labels for the Assay of Thrombopoietin (40686)[1]

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Mice have been utilized in several previous studies for the assay of a thrombocytopoiesis-stimulating factor (TSF or thrombopoietin). To measure platelet production rates, some workers (1–4) used [$^{75}$Se]selenomethionine ($^{75}$SeM) as a platelet label while others (5–11) used [$^{35}$S]sodium sulfate. Presumably, either isotope would label platelets to the same degree and could, therefore, be used to detect increased thrombocytopoiesis; however to date, the two isotopes have not been compared in the same study. The results show that both isotopes measure the increased thrombocytopoiesis that follows TSF injection, but $Na_2{}^{35}SO_4$ gives significantly greater values than $^{75}$SeM after high TSF doses when compared to suitable saline-treated control animals.

*Materials and methods.* Male C3H mice weighing approximately 25 g were used. Some of the mice were pretreated with a single intraperitoneal (i.p.) injection of rabbit anti-mouse platelet serum (RAMPS) which had been prepared and absorbed as previously described (7).

The TSF used in this study was culture medium from human embryonic kidney cells (Lot WTSF-80, 164 mg of protein/ml). Medium from kidney cell cultures was shown previously to contain high levels of TSF (12). The medium was collected, concentrated, and stored as previously described (11).

TSF was injected subcutaneously (s.c.) two times on Day 5 after RAMPS treatment and again two times on Day 6. On Day 7, either 30 $\mu$Ci (8) of [$^{35}$S]sodium sulfate (New England Nuclear, Boston, Mass.) or 1 $\mu$Ci (3) of [$^{75}$Se]selenomethionine (Sethotope, E. R. Squibb & Sons, New Brunswick, N. J.) diluted to 0.5 ml in saline was injected intravenously (i.v.) and the 24-hr isotope incorporation into platelets was measured. Using similar schedules, normal mice (mice not injected with RAMPS) were injected with TSF and radioisotopes.

Immediately before killing, mice were injected i.p. with 0.5 ml of heparin–Nembutal solution (heparin, 1000 U.S.P. units; Nembutal, 50 mg; 10 ml of saline). Platelet counts were taken from a single drop of blood obtained from the retroorbital sinus. Approximately 3 min later mice were bled (about 0.5 ml/mouse) by cardiac puncture into plastic syringes containing 1 ml of 1.0% EDTA, and the blood was expressed into plastic tubes. After dilution with additional EDTA solution to a total volume of 3.0 ml, the blood was centrifuged (4.5 min at 450 g) to obtain platelet-rich plasma. Platelet-rich plasma was removed and centrifuged (15 min at 800*g*) to obtain platelet "pellets"; the platelets were washed twice in 1.0% ammonium oxalate and once in saline. The washed platelets were resuspended into 0.35 ml buffered saline and the number of platelets in suspension was determined by use of an Electrozone/Celloscope (Particle Data, Inc.). Other samples of the platelet suspension were used to determine radioactivity. The % $^{35}$S and $^{75}$Se incorporation into the total platelet mass was calculated as previously described (7).

Student's *t* test was used to evaluate the results.

*Results.* Figure 1 shows the results of comparing $Na_2{}^{35}SO_4$ and $^{75}$SeM as platelet labels for measuring the amount of stimulation of thrombocytopoiesis in mice after injection of various doses of TSF-rich material. The two isotopes showed similar responses, i.e., as compared to saline-treated controls, TSF caused significantly more isotope incorporation into platelets of both normal and RAMPS-treated mice at all TSF dose levels. Isotope incorporation into platelets increased as the TSF doses were increased, indicating

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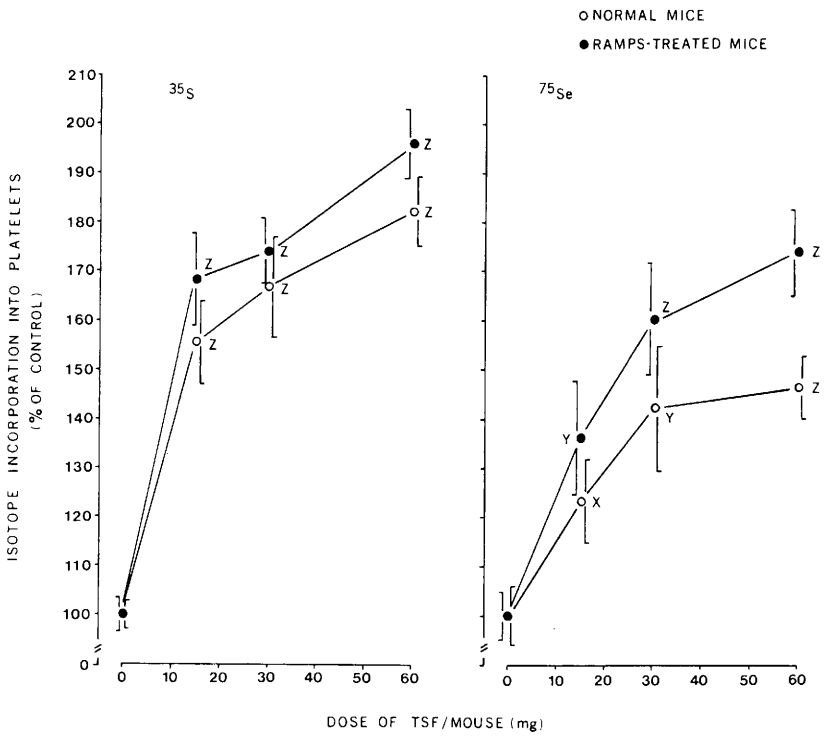


FIG. 1. Isotope incorporation into platelets of mice after injection with various doses of thrombopoietin (TSF)-rich kidney cell culture medium. Each point represents the average 8–35 mice and the vertical lines indicate the standard errors. $^{35}S$ ($Na_2$ $^{35}SO_4$) was injected at 30 $\mu$Ci/mouse and $^{75}Se$ ([$^{75}Se$]selenomethionine) was used at 1 $\mu$Ci/mouse. The % isotope incorporation into platelets was determined 24 hr later using blood obtained by cardiac puncture. Values were significantly different from control: saline-treated control mice versus TSF-injected mice: (*X*) $P < 0.05$, (*Y*) $P < 0.005$, (*Z*) $P < 0.0005$.

that all four groups of animals had positive dose–response curves.

As already noted, RAMPS-treated mice, because of lower baselines, showed greater responses than did normal mice. Although not shown, RAMPS pretreatment reduced the isotope incorporation into platelets of saline-treated mice: % $^{35}S$ incorporation into platelets of 35 normal mice was $4.26 \pm 0.13 \times 10^{-3}$ compared to $3.26 \pm 0.01 \times 10^{-3}$ for 29 RAMPS-treated mice; for $^{75}SeM$ the % incorporation into platelets of 30 normal mice was $5.16 \pm 0.31 \times 10^{-2}$ versus $4.03 \pm 0.23 \times 10^{-2}$ for 24 RAMPS-treated mice. As depicted in Fig. 1, the percentage incorporation of $^{35}S$ was greater at each TSF dose level than the % $^{75}Se$ incorporation, e.g., the difference between % $^{35}S$ incorporation and % $^{75}Se$ uptake was significant after injecting TSF at 60 mg/mouse for both normal mice ($P < 0.005$) and RAMPS-pretreated mice ($P < 0.05$).

*Discussion.* Several attempts have been made to find the optimum conditions for the assay of TSF (7–11). Mice in rebound thrombocytosis were more sensitive to exogenous TSF preparations than were normal mice. Also, if the first of four TSF injections was given on Day 5 after RAMPS treatment and the 24-hr %$^{35}S$ incorporation determined on Day 8, greater $^{35}S$ incorporation values were found than at the other times tested (11). The route of administering TSF did not seem to be important since TSF injected s.c. or i.p. gave essentially the same result (9). Multiple injections were more effective than single injections (9). Isotope incorporation gave a more sensitive measurement of thrombocytopoiesis than platelet counting or platelet sizing (8). Additional work (10) revealed that the time of measurement of $^{35}S$ incorporation into platelets and selection of the mouse strain determined to a large degree the

sensitivity of the TSF assay. Sex of the mice did not appear to be important; similar responses were found in both male and female mice (10). Our results show herein that although $^{35}S$ gave significantly greater values at the higher TSF doses, either [$^{35}S$]sodium sulfate or $^{75}SeM$ can be used to measure platelet production rates in mice stimulated with thrombopoietin.

For optimum results, great care should be used in selecting the mouse strain. The mice would be pretreated with platelet-specific antisera to make them thrombocytopenic (7–11). Five days later, when mice are thrombocytotic, they should be injected with test substances in multiple doses over a 2-day period, and platelet production rates measured by administering either $Na_2$ $^{35}SO_4$ or $^{75}SeM$; isotope incorporation into platelets should be measured 24 hr later (10).

*Summary.* Platelet production rates were measured in thrombopoietin (TSF) assay mice by use of both [$^{35}S$]sodium sulfate and [$^{75}Se$]selenomethionine ($^{75}SeM$) as platelet labels. Although significantly higher values were found using $Na_2$ $^{35}SO_4$ than $^{75}SeM$, the incorporation patterns for both isotopes showed similarities in dose–response experiments.

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1. Penington, D. G., Brit. Med. J. **1,** 606 (1970).
2. Nakeff, A., and Roozendaal, K. J., Acta Haematol. **54,** 340 (1975).
3. Evatt, B. L., Shreiner, D. P., and Levin, J., J. Lab. Clin. Med. **83,** 364 (1974).
4. Evatt, B. L., Spivak, J. L., and Levin, J., Blood **48,** 547 (1976).
5. Cooper, G. W., Cooper, B., and Chang, C.-Y., Proc. Soc. Exp. Biol. Med. **134,** 1123 (1970).
6. Cooper, G. W., Cooper, B., Ossias, A. L., and Zanjani, E. D., Blood **42,** 423 (1973).
7. McDonald, T. P., Proc. Soc. Exp. Biol. Med. **144,** 1006 (1973).
8. McDonald, T. P., Brit. J. Haematol. **34,** 257 (1976).
9. McDonald, T. P., Proc. Soc. Exp. Biol. Med. **155,** 4 (1977).
10. McDonald, T. P., Clift, R., and Cottrell, M., Exp. Hematol. **7,** 289 (1979).
11. McDonald, T. P., Clift, R., Nolan, C., and Tribby, I. I. E., Scand. J. Haematol. **16,** 326 (1976).
12. McDonald, T. P., Clift, R., Lange, R. D., Nolan, C., Tribby, I. I. E., and Barlow, G. H., J. Lab. Clin. Med. **85,** 59 (1975).

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