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Effects of Different Routes of Administration and Injection Schedules of Thrombopoietin on ^{35}S Incorporation into Platelets of Assay Mice (39732)[1]

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Mice have been previously used for the assay of a thrombocytopoiesis-stimulating factor (TSF or thrombopoietin). However, the procedures that have been used (1–8) differ markedly from one investigator to another. Normal mice (3–4, 6, 8), mice in rebound thrombocytosis (1, 2), or mice made thrombocytotic by platelet transfusion (5, 7) have been used. For TSF assays, both male (1, 2, 6) and female (4, 5) mice have been used; and each worker has used a different mouse strain. Also, mice have been injected subcutaneously, intravenously, or intraperitoneally with TSF-rich preparations and the percentage of either [^{75}Se]selenomethionine (^{75}SeM) or [^{35}S-]sodium sulfate incorporated into platelets has been measured as an index of platelet production. The present work compares the percentage of ^{35}S incorporation into platelets of mice in rebound thrombocytosis after sc and ip injections of TSF-rich materials by use of various injection schedules.

Methods. The TSF used in these studies was production culture medium from human embryonic kidney cells (Lot WTSF 75-C300) which had previously been shown to contain high levels of TSF, but did not contain erythropoietin, erythrogenin, or WBC-stimulating factors (9). Prior to storage, the production medium was concentrated (300 ×) by use of an Amicon Model LTCX-15-UM-10 ultrafiltration cartridge. The concentrate was then clarified by centrifugation at 10,000 *g* and 4° for 50 min and the supernatant fluid, with 258 mg of protein/ml, was stored at −76° until injected into assay mice. Control mice were injected with saline by use of the same injection schedule as was utilized for the TSF.

Ten- to twelve-week-old C_3H male mice were given a single ip injection of rabbit anti-mouse platelet serum (RAMPS) 5 days before injection of TSF-rich test materials. RAMPS, which had been prepared and absorbed with mouse RBC as previously described (2, 10), produced marked thrombocytopenia at 3–4 hr which was followed by rebound thrombocytosis 5–7 days later. Since previous results (7) established that the maximum response to TSF was obtained after injection of TSF on Days 5 to 6 and percentage of ^{35}S measurement on Day 8, these time intervals were used in the present studies.

In the first set of experiments, mice were injected four times either sc or ip with 0.5 ml of saline or TSF-rich material: two times on day 5 after RAMPS injection and then again two times on Day 6. $Na_2{}^{35}SO_4$ (30 μCi in 0.5 ml of saline) was injected iv on Day 7 and the percentage of ^{35}S incorporation into platelets was measured 24 hr later (Day 8) in blood samples obtained by cardiac puncture. Platelet counts were made on blood taken from the retroorbital sinus at the same time. The percentage of ^{35}S incorporation into the total platelet mass was calculated as previously described (2) using 8.3% of body weight as the blood volume.

In the second set of experiments, a dose-response relationship was determined for TSF-rich materials by injecting various amounts of medium (0–30 mg/mouse) diluted to 2.0 ml with saline. Mice were injected sc in four equal doses with the first two doses being given on Day 5 after RAMPS. $Na_2{}^{35}SO_4$ was administered as before on Day 7 after RAMPS injection, and the percentage of ^{35}S incorporation into platelets was determined 24 hr later (Day 8).

In the third set of experiments, all mice were injected sc with the same total amount of TSF-rich material (30 mg/mouse) using

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different dosage schedules, which are described in the legend to Fig. 2.

Student's t test was used throughout to evaluate differences in percentage of ^{35}S incorporation into platelets.

Results. Table I shows the effects of injecting TSF-rich materials sc and ip on percentage of ^{35}S incorporation into platelets of assay mice. Saline injected either sc or ip resulted in approximately the same percentage of ^{35}S incorporation values in assay mice 3 days after the first of four injections. However, TSF, given sc or ip, caused a significant ($P < 0.0005$) increase in percentage of ^{35}S incorporation into platelets when compared to saline-injected control mice. Moreover, no difference was observed between the two routes of administration of TSF. In all experiments to follow, mice were injected sc with TSF.

TABLE I. EFFECTS OF DIFFERENT ROUTES OF ADMINISTRATION OF A TSF-RICH PREPARATION ON PERCENTAGE OF ^{35}S INCORPORATION INTO PLATELETS OF ASSAY MICE.

Treatment[a]	Number of mice	^{35}S incorporation $\times 10^3 \pm$ SE (%)	Control (%)
Saline, sc	40	4.63 ± 0.19	–
TSF, sc	40	7.13 ± 0.29*	154
Saline, ip	19	4.76 ± 0.20	–
TSF, ip	14	7.59 ± 0.53*	159

[a] Thrombopoietin (TSF; 30 mg of WTSF 75-C300/mouse) or saline was injected sc or ip in four equal doses: two injections on Day 5 (one in the morning and one in the afternoon), and two injections on Day 6; ^{35}S was injected intravenously on Day 7 and the 24-hr percentage of ^{35}S uptake was measured on Day 8.

* Significantly higher than control, $P < 0.0005$.

The results of sc injections of various amounts of TSF (in four equal doses) to mice in rebound thrombocytosis are shown in Fig. 1. Increasing the dose of TSF caused greater incorporation of ^{35}S. The dose–response curve was linear ($Y = 100.5 + 1.94 X$).

Figure 2 shows the results of injecting mice sc with the same total dose (30 mg/mouse) of TSF, utilizing different injection schedules. The injection of saline, 1 or 2 ml

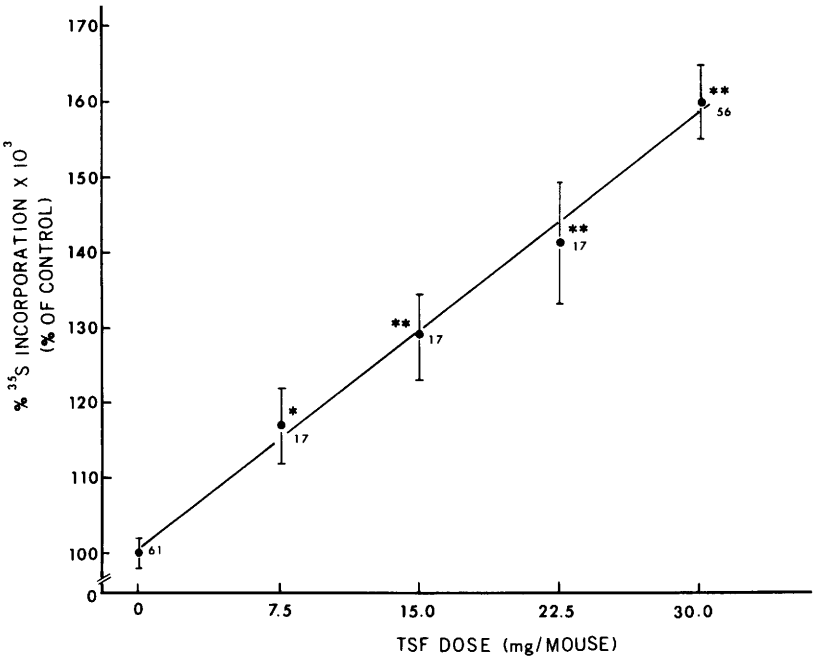

FIG. 1. The percentage of ^{35}S incorporation into platelets of assay mice after injection of various doses of TSF-rich culture medium. The numbers next to the points indicate the number of mice at each treatment level and the vertical bars indicate the standard error. The percentage of ^{35}S values were significantly higher than saline-injected control mice: (*)$P < 0.005$; (**) $P < 0.0005$. Mice were injected sc two times with 0.5 ml of TSF-rich medium diluted in saline on Day 5 after RAMPS injection; injections were repeated on Day 6 and [^{35}S]sodium sulfate was given on Day 7. Percentage of ^{35}S incorporation into platelets was determined 24 hr later.

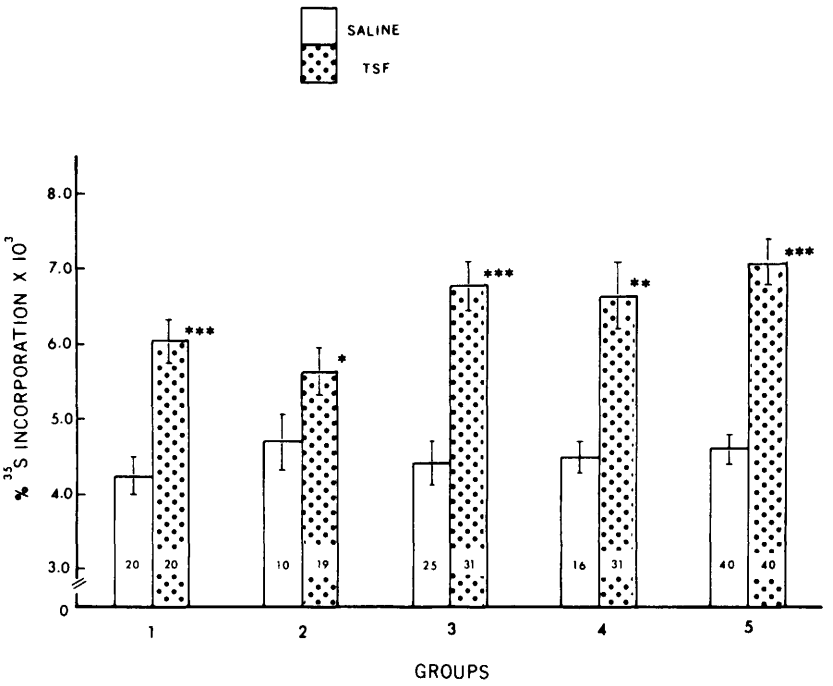

FIG. 2. The percentage of ^{35}S incorporation into platelets of assay mice after sc injections of TSF-rich culture medium. Numbers on bars represent the number of mice per treatment and the vertical lines indicate the standard error. The percentage of ^{35}S incorporation values for TSF-injected mice were significantly higher than for saline-injected control mice: (*)$P < 0.05$; (**)$P < 0.005$; (***)$P < 0.0005$. In all groups, mice were injected with the same total dose (30 mg/mouse) of TSF-rich material. Mice in group 1 were injected one time in the morning of Day 5 after RAMPS injection; mice in group 2 were injected one time on Day 6; mice in group 3 were injected two times, one 15-mg dose in the morning and one 15-mg injection in the afternoon on Day 5; mice in group 4 were injected two times on Day 6; and mice in group 5 were injected two times on Day 5 and then again two times on Day 6. $Na_2{}^{35}SO_4$ was given on Day 7 and the percentage of ^{35}S incorporation into platelets was determined 24 hr later.

over a 2-day period, did not significantly ($P > 0.10$) change the percentage of ^{35}S incorporation into platelets of assay mice. In all groups, the injection of TSF caused significant ($P < 0.05$ to 0.0005) increases in percentage of ^{35}S incorporation when compared with suitable controls. However, four injections of TSF (group 5) gave higher ^{35}S uptakes than one injection on Day 6 (group 2, $P < 0.005$) or one injection on Day 5 (group 1, $P < 0.025$). Although not statistically significant, either one or two TSF injections given on Day 5 (group 1 and 3) resulted in higher ^{35}S values than did injections given on Day 6 (groups 2 and 4). Likewise, a slightly better response was obtained with multiple injections than with single injections of the same total amount of TSF.

Discussion. Previously, TSF-rich preparations have been injected sc (1–3, 7, 8), iv (6), or ip (4, 5). The present study compared both sc and ip injections of TSF-rich preparations (Table I), and showed that both routes of administration of TSF gave approximately the same percentage of ^{35}S incorporation into platelets of assay mice. Therefore, future TSF assays could utilize either route of administration with equally good results.

TSF has been previously administered in two or four injections over a period of 48 hr (1–4, 6, 8) or six injections over 72 hr (1, 5) before administration of radioisotopes. Some investigators have used 16 (3, 8), 24 (1), 48 (1, 6), and 72-hr (1) ^{75}SeM incorporation into platelets for TSF assay. Other workers gave $Na_2{}^{35}SO_4$ and measured per-

centage of ^{35}S incorporation after 24 (2) or 48 hr (4, 5). A significant dose–response relationship was observed in the present study after four injections of various doses of TSF-rich culture medium and measurement of 24-hr ^{35}S incorporation (Fig. 1).

Only Penington (1) has previously investigated the effect of different injection schedules of TSF-rich materials on isotopic incorporation into platelets of assay mice. He quantitated the effects of TSF injections on Days 7 to 9 after antisera injection and showed that TSF administered over a 2-day period with four injections gave the greatest ^{75}SeM incorporation values. The results of the present work indicate that injection of TSF one or two times on Day 5 after RAMPS injection, or in four doses over a 2-day period (with the same total dose), gave higher ^{35}S uptakes (Fig. 2) than did one or two injections of TSF given on Day 6. The findings of the present report, therefore, agree with those of Penington (1) and show that, for best results, TSF-rich materials should be administered either sc or ip in multiple injections over a period of about 48 hr before isotope injection.

Summary. The 24-hr percentage of ^{35}S incorporation into platelets of mice in rebound thrombocytosis was compared after single or multiple sc and ip injections of a thrombopoietin (TSF)-rich preparation. TSF administered sc or ip resulted in almost identical percentage of ^{35}S incorporation values. Various doses of TSF injected sc two times on Day 5 after RAMPS and two times again on Day 6 gave a linear dose–response curve. Also, this injection schedule gave the greatest response to the same total dose of TSF when compared to other injection schedules. Single injections of TSF did not give as great a response as did multiple injections using the same total dose. Results of this work indicate that for maximum percentage of ^{35}S incorporation values, TSF-rich materials should be administered in multiple injections to mice, either sc or ip, over a period of approximately 2 days.

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