# Cortisone Action on Serum Colony-Stimulating Factor and Bone Marrow *in Vitro* Colony-Forming Cells* (34222)

DONALD METCALF
(Introduced by E. A. Mirand)
*Cancer Research Unit, Walter and Eliza Hall Institute, Royal Melbourne Hospital, Australia*

Hemopoietic precursor cells in mouse bone marrow can proliferate in semisolid agar cultures and form colonies of granulocytic and/or macrophage cells, provided that the cultures are stimulated by a factor known as the "colony-stimulating factor" (CSF) (1–3). The CSF is detectable in the serum of most normal mice but levels vary widely in individual, apparently healthy, mice (1, 3). Serum CSF levels in mice are elevated by acute infections, leukemia, antigenic stimulation, and irradiation (1, 3–5) and are very low in germfree mice (6). Many of these situations involve stress to the animal, and because of this, the present experiments were undertaken to determine the effects of cortisone on serum CSF levels and on bone marrow content of *in vitro* colony-forming cells.

*Methods.* Experimental mice were 6-month-old C57BL mice of both sexes. The technique of agar culture of mouse bone marrow cells and the media used have been described elsewhere (1, 3). Modifications to the technique in the present experiments were the use of 10% $CO_2$ in air in the incubator and the supplementation of the medium by L-asparagine (final concentration 20 $\mu$g/ml) and DEAE-dextran (final concentration 75 $\mu$g/ml). Sera were assayed for colony stimulating activity in duplicate 1 ml cultures using 0.1- and 0.05-ml doses and 75,000 bone marrow cells from 2-month-old C57BL mice as target cells. Colonies were scored at 7 days and serum CSF levels expressed as the mean number of colonies stimulated by 0.1 ml of serum. Assays in individual mice for the bone marrow content of *in vitro* colony-forming cells were performed as described previously (7) using replicate 1-ml cultures containing 50,000 bone marrow cells and 0.1 ml of mouse serum or 0.15 ml of human urine concentrate of known colony-stimulating activity (8) to stimulate colony formation. Bone marrow cell suspensions for culture were obtained from one femur shaft and total shaft cell counts were performed on the other femur. Since the number of colonies developing *in vitro* per $10^5$ bone marrow cells is dependent in part on the concentration of CSF in the cultures (1, 3), estimates of colony-forming cells in individual experimental mice were expressed as a percentage of mean levels in control, saline-injected, mice in the same assay run. Cortisone acetate (Merck) was injected subcutaneously as a suspension in normal saline of constant volume, 0.2 ml. Control mice were saline injected.

*Results. Effect on serum colony-stimulating activity.* Following the subcutaneous injection of 2 mg of cortisone acetate, serum colony-stimulating activity fell sharply (Fig. 1) to 10% of control serum levels by 6 hr. Serum activity remained low for 2 days following a single injection but thereafter rose and reached control levels by 7 days after injection. Those colonies which were stimulated to develop by sera from cortisone-treated mice appeared normal in size and gross morphology and contained similar populations of granulocytic and/or macrophage cells to those present in colonies stimulated to develop by control sera. An analysis of the effects at 6 hr of single injections of varying doses of cortisone on serum colony-stimulating activity revealed (Fig. 2) that doses as low as 0.25 mg had a significant

* This work was supported by the Carden Fellowship Fund of the Anti-Cancer Council of Victoria.

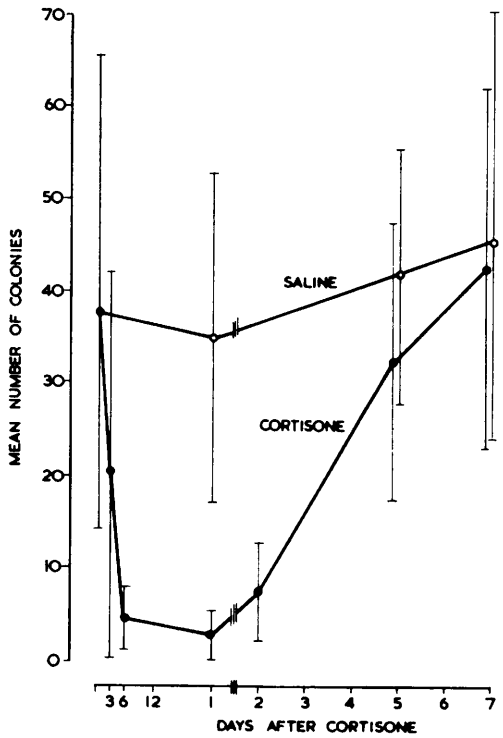


FIG. 1. Serum colony-stimulating activity in mice injected with cortisone or saline, expressed as mean number of colonies stimulated by 0.1 ml of serum; each point represents mean data from 10 mice, and vertical bars are the standard deviations of these values.

depressing effect. Mice injected with 2 mg of cortisone developed a profound polyuria, excreting an average of 6 times the volume of urine that saline-injected mice excreted over the 6-hr period following injection. The confinement of even uninjected mice in metal urine collection cages for 24 hr in a cool room resulted in stress to the animals as judged by general appearance. A group of twenty, 6-month-old C57BL mice were kept in collection cages for 24 hr and compared with 20 control mice. Mean thymus weights were: control 39 $\pm$ 6 mg; stressed 29 $\pm$ 6 mg ($t$ 4.9; $p<0.01$). Serum colony-stimulating activity: control 32$\pm$10 colonies/0.1 ml; stressed 8 $\pm$ 5 colonies/0.1 ml ($t = 9.3$; $p<0.01$). The results indicate that stress, probably by affecting endogenous cortisone secretion, can lower serum CSF levels by measurable amounts.

Evidence was sought for the presence of an inhibitor in serum from cortisone-treated mice, which might either mask true levels of CSF or be toxic for colony-forming cells. Individual sera from 12 mice treated 6 hr previously with 2 mg of cortisone were tested alone or mixed with an equal volume of pooled normal C57BL sera of known activity, and tested for colony-stimulating activity. The results indicated no apparent inhibitory activity of the cortisone-treated sera on the colony-stimulating activity of normal serum (mean number of colonies stimulated by 0.05 ml of cortisone-treated sera, 0.6 $\pm$ 0.4; 0.05 ml of pooled normal serum, 46 $\pm$ 9; 0.05 ml of cortisone-treated serum + 0.05 ml of pooled normal serum, 51 $\pm$ 16).

*Effect on bone marrow colony-forming cells.* Sixty-one mice were injected with 2 mg of cortisone acetate and 61 control mice with saline. Assays of *in vitro* colony-forming cells were performed on these mice at intervals after injection, and two changes developed (Fig. 3). The total cell count per femur shaft rose to a maximum level of 40% above that in control mice at 3 days after cortisone. Synchronous with this rise in cellularity was a progressive fall in the frequency of colony-forming cells compared with levels in saline-

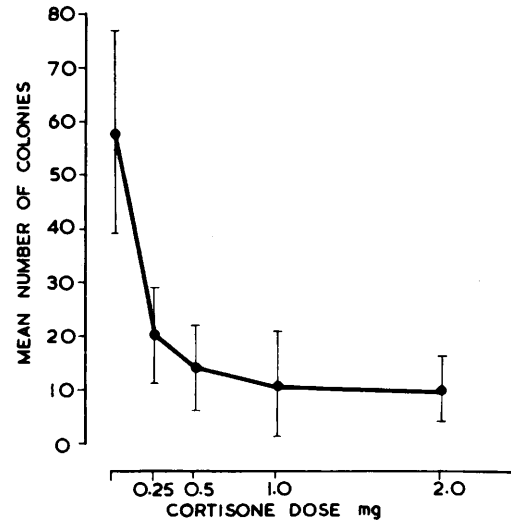


FIG. 2. Serum colony-stimulating activity 6 hr after the injection of varying doses of cortisone; each point represents mean data from 10 mice, and vertical bars are the standard deviations of these values.

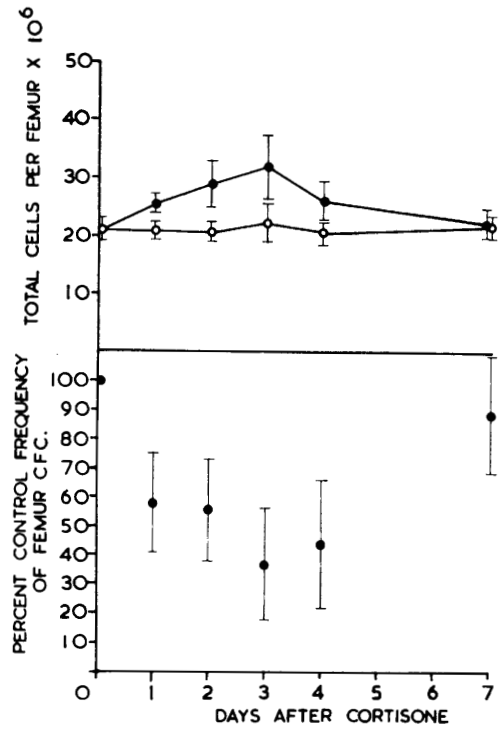


FIG. 3. Effects of 2 mg of cortisone on bone marrow cellularity and frequency of *in vitro* colony-forming cells; each point, mean values of 8–15 mice.

injected control mice, reaching minimum values at day 3 after injection. The fall in frequency of colony-forming cells was more marked than the rise in total cell count, indicating that a fall in absolute numbers of *in vitro* colony-forming cells of approximately 40% had occurred by day 3 after cortisone injection. Bone marrow cellularity and colony-forming cell content had returned to normal levels by day 7.

The addition of cortisone acetate to 1-ml cultures of 75,000 normal C57BL bone marrow cells inhibited urine-stimulated colony formation when the dose per culture was 10 $\mu$g or higher (Table I). Assays at 7 days for CSF in negative cultures to which 100 $\mu$g of cortisone had been added originally, indicated that the cortisone had not inactivated the added urine CSF and presumably had inhibited colony formation by a direct toxic effect on colony-forming cells.

*Discussion.* A single injection of cortisone produced an acute fall in serum colony-stimulating factor (CSF) levels and a more slowly developing fall in the bone marrow content of *in vitro* colony-forming cells. These two changes are not necessarily cause and effect and both may represent independent effects of cortisone. In this regard, cortisone appeared to inhibit colony formation *in vitro*, possibly by a direct toxic effect on colony-forming cells.

The doses of cortisone inhibiting colony formation *in vitro* (10 $\mu$g/g) are less than those which have been used clinically (9) in the treatment of acute leukemia (70 $\mu$g/g) and indicate one probable effect of cortisone on the bone marrow of such patients. A similar comment can be made on the cortisone doses found to significantly depress serum CSF levels (8 $\mu$g/g).

The mechanism of the fall induced by cortisone in serum CSF levels has not been established. It is possible that an inhibitor develops in cortisone-treated mice which inactivates CSF in the serum. No evidence for such an inhibitor was obtained but the present experiments would only have detected excess levels of inhibitor, not inhibitor bound to factor. Cortisone-treated mice developed a profound diuresis and as CSF is known to be excreted in large amounts in the urine (8), this suggests that serum factor levels may fall due to excessive loss via the

TABLE I. Inhibition by Cortisone of Colony Formation *in Vitro* by Bone Marrow Cells.

| Dose of cortisone per culture ($\mu$g)[a] | Mean percentage inhibition of colony formation[b] |
|---|---|
| 100 | 100 |
| 75 | 100 |
| 50 | 99 |
| 25 | 93 |
| 10 | 36 |
| 1 | 7 |
| 0.1 | 8 |
| 0.01 | 0 |

[a] Constant volume (0.1 ml) of cortisone acetate added to each culture (1 ml); colony formation stimulated by 0.1 ml of human urine concentrate.

[b] Colony counts performed on day 7; data from two separate experiments, using four replicate cultures at each dose.

urine. In this regard, preliminary experiments (Chan, S. H., unpublished data) have indicated that cortisone does not cause a fall in serum CSF levels in nephrectomized mice.

The present results indicate the need for caution in interpreting the effects of any procedure involving stress on serum CSF levels or bone marrow *in vitro* colony-forming cell levels, since stress-induced endogenous cortisone production may produce significant changes.

*Summary.* Cortisone acetate in adult C57BL mice, in doses of 0.25–2.0 mg, caused an acute fall in serum levels of colony-stimulating factor and a slower fall in the bone marrow content of *in vitro* colony-forming cells reaching minimum values 3 days after injection. Doses of cortisone as low as 10 $\mu$g inhibited colony formation *in vitro* by bone marrow cells.

1. Robinson, W., Metcalf, D., and Bradley, T. R., J. Cell Physiol. **69**, 83 (1967).

2. Metcalf, D., Bradley, T. R., and Robinson, W., J. Cell Physiol. **69**, 93 (1967).

3. Metcalf, D. and Foster, R., J. Natl. Cancer Instit. **39**, 1235 (1967).

4. Foster, R., Metcalf, D., and Kirchmyer, R., J. Exptl. Med. **127**, 853 (1968).

5. Hall, B., Brit. J. Haematol. (1969), in press.

6. Metcalf, D., Foster, R., and Pollard, M., J. Cell. Physiol. **70**, 131 (1967).

7. Metcalf, D., Brit. J. Haematol. **16**, 397 (1969).

8. Metcalf, D. and Stanley, E. R., Australian J. Exptl. Biol. Med. **47**, 453 (1969).

9. Bernard, J., Bilski-Pasquier, G., and Deltour, G., Bull. Soc. Med. Hop. Paris **70**, 579 (1954).

Received June 6, 1969. P.S.E.B.M., 1969, Vol. 132.

## ERRATUM

Vol. **131**, No. 2 (1969), in the article, "Excretion of Exogenous Creatinine by the Guinea Pig Kidney," by Richard J. Laurence and Ewald E. Selkurt, pp. 550-554:

Page 550, the word "Creatine" in the title should read "Creatinine."