[4] Fillenz, M., *Proc. Roy. Soc.*, B, **174**, 459 (1970).
[5] Laverty, R., and Taylor, K. M., *Anal. Biochem.*, **22**, 269 (1968).
[6] Viveros, O. H., Arqueros, L., and Kirshner, N., *Life Sci.*, **7**, 609 (1968).
[7] Hvidt, A., Johansen, G., Linderström-Lang, K., and Vaslow, F., *CR Trav. Lab. Carlsberg, Ser. Chim.*, **29**, 129 (1954).
[8] Smith, A. D., De Potter, W. P., and De Schaepdryver, A. F., *Arch. Intern. Pharmacodyn.*, **179**, 495 (1969).
[9] Hörtnagl, H., Hörtnagl, H., and Winkler, H., *J. Physiol.*, **205**, 103 (1969).
[10] Roth, R. H., Stjärne, L., Bloom, F. E., and Giarman, N. T., *J. Pharmacol. Exp. Therap.*, **162**, 203 (1968).

Effect of Steroids on the Cycling of Haemopoietic Stem Cells

THIS report describes an effect (not previously reported) of single doses of steroids on the cell cycle of haemopoietic stem cells. This effect, one of increasing the sensitivity of spleen colony forming units (CFU) to the lethal effects of ^{3}H-thymidine, is seen well before the long-term effects of steroids on erythropoiesis[1]. Increased sensitivity of CFU to ^{3}H-thymidine was also observed in steroid-treated polycythaemic mice. The latter finding suggests that the steroid effect is not mediated through erythropoietin.

Normal or polycythaemic $C_3H \times AKR$ female mice were used in these experiments; they were 9–11 weeks old and weighed 25 ± 2 g. To suppress endogenous levels of erythropoietin[2], mice were made polycythaemic by the method of hypertransfusion. The technique used in these experiments has been described previously[3]. Testosterone propionate (TP), etiocholan-17β-ol-3-one (ETIO), 19-nortestosterone phenylpropionate (19-nor-TPP), and 5α-androstane (5α-AND) were the steroids studied. A 1·8 per cent solution of medium viscosity carboxymethylcellulose (CMC) in physiological saline was used to suspend the compounds. Suspensions of steroids were 0·058 mmole/ml. The agents were injected subcutaneously in a single dose of $2{\cdot}9 \times 10^{-4}$ mmole/g of mouse; this was equivalent to 0·10 mg/g TP, 0·084 mg/g ETIO, 0·118 mg/g 19-nor-TPP, and 0·076 mg/g 5α-AND.

TP was the only steroid studied in polycythaemic mice. To test whether TP causes an increase in endogenous erythropoietin levels, TP-treated polycythaemic mice were injected with 0·5 μCi ^{59}Fe 54 h after steroid. Incorporation of ^{59}Fe into red blood cells was measured 72 h later[3]. This was compared with ^{59}Fe incorporation in control polycythaemic mice.

CFU were estimated using the method of Till and McCulloch[4]. 6 h after treatment with either CMC or steroids, bone marrow was collected from the femurs of two to four mice and pooled; the cells were suspended in 2·0 ml./femur of cold Fischer's medium. Sensitivity to ^{3}H-thymidine[5] was determined by incubating cell suspensions ($2{\cdot}5$–$4{\cdot}5 \times 10^6$ cells/ml.) *in vitro* at 37° C for 30 min with or without 200 μCi/ml. of high specific activity (13·4–22·8 Ci/mmole) ^{3}H-thymidine. After incubation the cell suspensions were adjusted to 2×10^5 cells/ml. with cold Fischer's medium. 5×10^4 cells were injected intravenously to mice (10–12 mice/group) previously given 850 rad total-body X-irradiation. Spleen colonies were counted 9–10 days after bone marrow transplantation. Sensitivity to ^{3}H-thymidine was expressed as the per cent loss of CFU from cell suspensions incubated with ^{3}H-thymidine:

$$^3\text{H-thymidine effect (per cent)} = 100 - \left[\frac{\text{number of CFU with } ^3\text{H-thymidine}}{\text{number of CFU without } ^3\text{H-thymidine}} \times 100\right]$$

The technique estimates, at best, the minimum per cent of CFU in DNA synthesis and therefore in cell cycle.

Table 1 gives the effects of ^{3}H-thymidine on bone marrow from CMC and steroid treated mice. CFU from CMC and 5α-AND treated mice were insensitive to ^{3}H-thymidine. An increased sensitivity is shown for CFU from mice treated with either TP, ETIO, or 19-nor-TPP. Table 2 gives the results in polycythaemic animals. TP increased the sensitivity of CFU to ^{3}H-thymidine without increasing the incorporation of ^{59}Fe into red blood cells.

Table 1. EFFECT OF ^{3}H-THYMIDINE 6 H AFTER STEROID (NORMAL MICE)

Treatment	CFU/5×10^4 cells Without ^{3}H-thymidine	With ^{3}H-thymidine	^{3}H-thymidine effect (per cent)
CMC	$9{\cdot}8 \pm 0{\cdot}8$	$10{\cdot}1 \pm 1{\cdot}0$	0
5α-AND	$9{\cdot}8 \pm 1{\cdot}0$	$9{\cdot}7 \pm 0{\cdot}5$	1·1
TP	$9{\cdot}2 \pm 0{\cdot}9$	$6{\cdot}3 \pm 0{\cdot}5$	31·5
ETIO	$10{\cdot}3 \pm 0{\cdot}8$	$7{\cdot}0 \pm 0{\cdot}7$	32·1
19-nor-TPP	$13{\cdot}1 \pm 1{\cdot}2$	$10{\cdot}3 \pm 1{\cdot}0$	21·4

Table 2. EFFECT OF ^{3}H-THYMIDINE 6 H AFTER TP (POLYCYTHAEMIC MICE)

Treatment	CFU/5×10^4 cells Without ^{3}H-thymidine	With ^{3}H-thymidine	^{3}H-thymidine effect (per cent)	^{59}Fe incorporation*
CMC	$10{\cdot}6 \pm 0{\cdot}5$	$11{\cdot}0 \pm 0{\cdot}8$	0	0·20
TP	$11{\cdot}7 \pm 0{\cdot}5$	$8{\cdot}8 \pm 0{\cdot}5$	24·8	0·19

* Expressed as percentage injected dose of ^{59}Fe.

These results demonstrate an effect of steroids, 6 h after injection, on the cycling of stem cells from mouse bone marrow. CFU of adult mouse bone marrow are insensitive to the killing action of ^{3}H-thymidine[5]; for example, CMC group (Table 1). It is therefore assumed that normally CFU are not in cell cycle (that is, in a resting or G_0 stage), or have very long cell cycles. The results suggest that steroids may act to trigger haemopoietic stem cells into cell cycle or to shorten their cell cycles. The mechanism by which steroids might trigger CFU into cycle must await future studies. The activity of TP in polycythaemic mice (Table 2) indicates, however, that the steroid action does not require the presence of erythropoietin. One may speculate that protein synthetic mechanisms at the level of transcription or translation are involved[6].

The effectiveness of ETIO and 19-nor-TPP on the cycling of CFU indicates that steroids with potent androgen activity, for example TP, are not required for increasing DNA synthesis in haemopoietic stem cells. In addition, the results with ETIO demonstrate that steroids with a 5β configuration (A/B rings are *cis*) may be active in the haemopoietic system. These findings are in agreement with those of others who have demonstrated the activity of steroids with the 5β configuration on other components of the haemopoietic system[7–9].

Repopulation of the haemopoietic system, for example after damage by ionizing radiation or chemotherapeutic agents, depends, in part, upon the active proliferation of haemopoietic stem cells; that is, CFU. As proliferation may not proceed immediately following damage, the triggering of CFU into cycle immediately after haemopoietic damage may lead to an earlier recovery of the haemopoietic system. Preliminary studies indicate that TP given 1 h and 24 h after 150 rad total body X-irradiation does lead to more rapid recovery of the CFU population[10].

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[1] *Ann. NY Acad. Sci.*, **149** (Part VI), 298 (1968).
[2] Jacobson, L. O., Goldwasser, E., and Gurney, C. W., *Haemopoiesis* (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 423 (Churchill, London, 1960).
[3] Byron, J. W., and Lajtha, L. G., *Brit. J. Haematol.*, **15**, 47 (1968).
[4] Till, J. E., and McCulloch, E. A., *Radiat. Res.*, **14**, 213 (1961).
[5] Becker, A. J., McCulloch, E. A., Siminovitch, L., and Till, J. E., *Blood*, **26**, 296 (1965).
[6] Karlson, P., *Persp. Biol. Med.*, **6**, 203 (1963).
[7] Kappas, A., and Granick, S., *J. Biol. Chem.*, **243**, 346 (1968).
[8] Gorshein, D., and Gardner, F. H., *Proc. US Nat. Acad. Sci.*, **65**, 564 (1970).
[9] Gordon, A. S., Zanjani, E. D., Levere, R. D., and Kappas, A., *Proc. US Nat. Acad. Sci.*, **65**, 919 (1970).
[10] Byron, J. W., and Testa, Nydia, *Thirteenth Intern. Cong. Haematol.* (1970).