Response of Diploid, Heteroploid, and Viral-Transformed Animal Cell Cultures to Dinabol (Methandrostenolone) (33570)

E. MIEDEMA[1], W. WHITTLE, AND P. F. KRUSE, JR.
Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma 73401

Growth promoting effects of androgenic steroids were known as early as 1935 and this property has since found use clinically. Prominent among the effects of androgenic hormones are reduction of nitrogen loss and stimulation of muscle protein synthesis. Thus, it occurred to us that perhaps in tissue cultured cells a differential response to Dianabol (methandrostenolone), an anabolic androgenic steroid, might occur among diploid and viral-transformed connective tissue cells and between embryonic rat muscle cells and rat sarcoma cells. The only other known reported effects of Dianabol on animal cells *in vitro* did not evaluate this possibility but rather tested the effects of Dianabol on interferon production (1, 2). This study revealed an unexpected finding that carcinoma and sarcoma cell proliferation was markedly inhibited by Dianabol, whereas their diploid and viral-transformed counterparts were not.

Materials and Methods. Dianabol was generously supplied by CIBA Pharmaceutical Company, Summit, N. J. Human diploid WI-38 and viral-transformed WI-38VA13A cells were obtained from The Wister Institute, Philadelphia, Pa. The FLOW-2000 diploid human fibroblasts were secured from Flow Laboratories, Inc., Rockville, Md. Embryonic rat muscle and Jensen sarcoma cells, as well as human diploid JAM cells (derived from tonsil tissue from an 8-year-old male) were initiated from stock cultures in this laboratory. All cells were maintained and tested in medium 7a + 10% whole calf serum (3). Several experiments were run on each of the 6 cell types, with the majority of tests employing initial inocula of from 1.3 to 8.5 $\times$ 10^5 cells/T-25 flask. A few tests were made with higher inocula, up to 27 $\times$ 10^5 cells/T-25. Twenty-four hr after subcultures, the sticking cells were in the exponential phase of proliferation and were replenished with fresh, prewarmed medium 7a or 7a plus 0.25, 2.5, 5.0, 10.0, and 25.0 μg/ml of Dianabol. Tests were conducted for 72 hr, with medium changes at 48 hr when needed.

Periodic examination of stock cultures for mycoplasma were negative. Cell proliferation was measured by taking whole cell counts with a hemocytometer. Protein was assayed by the procedure of Oyama and Eagle (4). Uptake of radioactive lysine-^{14}C and uridine-^{3}H was determined by liquid scintillation counting procedures (5).

Results and Discussion. The proliferative response of Jensen sarcoma rat cells compared to embryonic rat muscle cells at increasing concentrations of Dianabol is shown

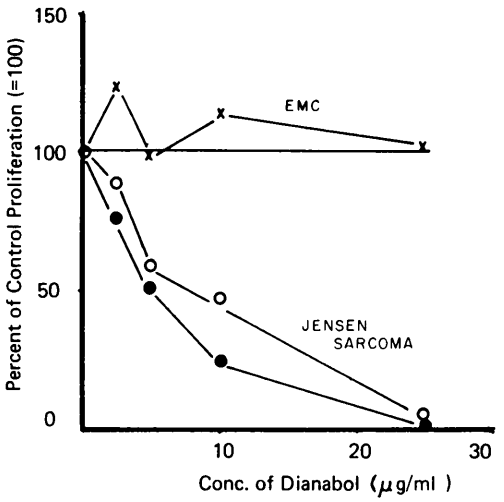

FIG. 1. Proliferative response of rat cells to Dianabol. (×), rat embryonic muscle cells (EMC); (○) and (●), two rat Jensen sarcoma cell cultures tested at 6-month intervals before (●) and after (○) being transplanted intramuscularly in host rats. Cell numbers were determined by hemocytometer counts. Initial inocula of control EMC and Jensen cultures increased cell numbers up to 9.3- and 12.4-fold, respectively, in 72 hr.

[1] Present address: Southern State College, Springfield, South Dakota 57602.

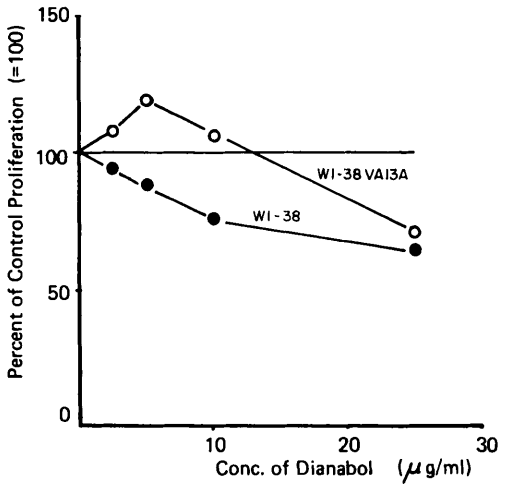

FIG. 2. Response of WI-38 diploid (●) and WI-38VA13A viral-transformed (○) human fibroblast cultures to Dianabol. Cell numbers were determined by hemocytometer counts. Initial inocula of control WI-38 and VA13A cultures increased cell numbers up to 7.9- and 8.1-fold, respectively, in 72 hr.

in Fig. 1. The response by embryonic rat cells was somewhat variable but in no case did Dianabol significantly inhibit proliferation over the concentration range tested (0–25 μg/ml). In the case of Jensen sarcoma rat cells, however, as little as 0.25 μg/ml of Dianabol had noticeable inhibitory effects on cellular proliferation. At the highest concentration tested (25 μg/ml), complete inhibition of proliferation was attained. Inhibition persisted in the Jensen cells when tested again at a much later date and after being carried as a transplantable tumor intramuscularly in rats. A semilog plot of the Jensen data indicated that the inhibition was linear on a log scale to increasing concentrations of the drug.

The effect of Dianabol on proliferation of diploid and viral-transformed human fibroblast cells is shown in Fig. 2. Although both cell lines were inhibited slightly by high concentrations of Dianabol, the degree of inhibition was markedly less than that observed for the sarcoma cells above. Unlike the sarcoma cells, the viral-transformed cells were less affected by the drug than their diploid counterpart.

Because of the noninhibitory effect of Dianabol on parent and/or viral-transformed human cells, it was next tested on a carcinoma cell line and another male and female human diploid cell. As shown in Fig. 3, only the carcinoma cell (HEp-2) was inhibited by Dianabol at the concentrations tested. The FLOW-2000 cells are female human diploid fibroblasts and the JAM cells are male diploid fibroblasts. Neither the FLOW-200 nor the JAM cells were inhibited, negating a possible sex difference in cellular sensitivity to proliferation inhibition by Dianabol in this study. The carcinoma cells (HEp-2), like the sarcoma cells (Jensen), showed a linear log-dose inhibition by the drug.

The effect of Dianabol (10 μg/ml) on protein synthesis in 4 of these cell types was tested by measuring the amount of lysine-^{14}C incorporated in 30 min/1 mg of culture protein. The results are summarized in Table I. The effect of Dianabol on lysine-^{14}C uptake was determined on 0 hr cultures and on cells grown in the presence of the drug (10 μg/ml) for 48 hr. In both cases uptake of lysine-^{14}C into cell protein was not decreased to a degree that could be correlated with the prolifer-

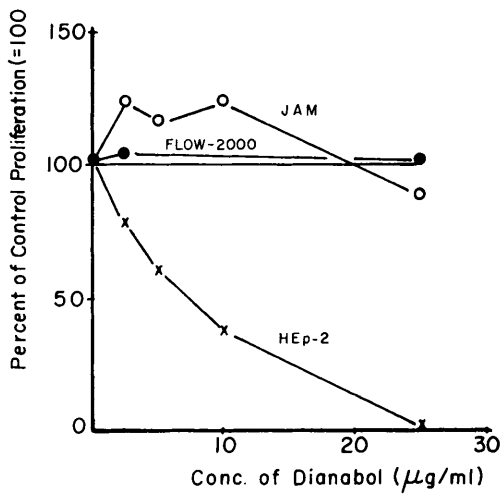

FIG. 3. Response of JAM male (○) and FLOW-2000 female (●) diploid human fibroblasts and HEp-2 human carcinoma (×) cells to increasing concentrations of Dianabol. Cell numbers were determined by hemocytometer counts. Initial inocula of control JAM, FLOW-2000, and HEp-2 cultures increased cell numbers up to 5.7-, 2.6-, and 10.5-fold, respectively, in 72 hr.

TABLE I. Effect of Dianabol on Protein Synthesis in Animal Cell Cultures.

Cell line and age (hr) of culture when tested	Lysine-^{14}C uptake (dpm/30 min/mg of culture protein)	
	Control	Dianabol[a]
WI-38		
0	3690	3310 (90)[b]
48[c]	4506	4145 (92)
VA13A		
0	5205	4967 (95)
48	6915	5495 (79)
HEp-2		
0	6900	6900 (100)
48	6578	5581 (85)
Jensen		
0	6983	6647 (95)
48	5282	4183 (79)

[a] Dianabol was tested at 10 μg/ml in all cases.

[b] Numbers in parentheses represent percentage of control values.

[c] Cells were grown for 48 hr in control medium or in medium supplemented with Dianabol at 10 μg /ml.

ation inhibition caused by Dianabol in the sarcoma and carcinoma cells.

The RNA synthesis, tested by incorporation of uridine-^{3}H, was likewise not inhibited by Dianabol (10μg/ml) in either WI-38 or HEp-2 cells; thus, it was not investigated further.

The inability of Dianabol to alter the protein synthetic ability of cells that are markedly inhibited in their proliferation may be explained in one of several ways. Dianabol's primary effect, like hydrocortisone, may be on the cell membranes (6, 7). A second plausible explanation is that the tumor cells are insensitive to processes regulating protein synthesis of diploid cells *in vitro* as previously shown for the Jensen sarcoma (8). A third alternative is that the ability of cells to take up the drug from the medium and/or convert it to an active form capable of affecting protein synthesis is lost *in vitro*. This limiting ability of the cells to be affected by the drug may be in part a reflection of the low cell densities encountered herein vs high cell densities optimal for collagen synthesis by cultured cells (9). The present results also suggest that carcinoma and sarcoma cells may be set apart from viral-transformed cells by their sensitivity to androgenic drugs *in vitro*.

Summary. An anabolic androgenic steroid, Dianabol (methandrostenolone), caused marked inhibition of proliferation when added to carcinoma and sarcoma cell cultures at concentrations of 0.25–25 μg/ml. Similar concentrations of the drug were noninhibitory when added to cultures of diploid, embryonic, and viral-transformed male or female fibroblasts. Protein and RNA synthesis, measured by uptake of lysine-^{14}C and uridine-^{3}H, was unaltered by the Dianabol in the sensitive as well as the insentive cell types.

The authors thank R. Harris and J. Nash for their technical assistance.

1. De Maeyer, E. and De Maeyer, J., Nature **197** 724 (1963).

2. Reinicke, V., Acta Pathol. Microbiol. Scand. **64**, 167 (1965).

3. White, P. B., Smith, C. W., and Kruse, P. F., Jr., Cancer Res. **23**, 1051 (1963).

4. Oyama, V. I. and Eagle, H., Proc. Soc. Exptl. Biol. Med. **91**, 305 (1956).

5. Bruno, G. A. and Christian, J. E., Anal. Chem. **33**, 1216 (1961).

6. Melnykovych, G., Science **152**, 1086 (1966).

7. Polet, H., Exptl. Cell Res. **41**, 316 (1966).

8. Kruse, P. F., Jr., Miedema, E., and Carter, H. C., Biochemistry **6**, 949 (1967).

9. Kruse, P. F., Jr. and Miedema, E., J. Cell Biol. **27**, 273 (1965).

Received Oct. 4, 1968. P.S.E.B.M., 1969, Vol. 130.