except one. Slope was changed in only 2 tests, one each in rats and mice but not with the same compound.

We wish to thank Mr. Charles Davies for valuable technical assistance.

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In vitro Culture of IRC 741 Rat Leukemia.* (30575)

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IRC 741 rat leukemia, also known as Dunning leukemia, is an acute leukemia that occurred spontaneously in a Fischer line 344 female rat bearing the transplanted acetylaminofluorene-induced mammary adenocarcinoma IRC 741(1), and has since been carried *in vivo* in various laboratories. The pathophysiological features of the leukemia have been described(2), and several reports on its use in screening chemotherapeutic agents have appeared(3-8).

We felt that it would be of interest to perform biochemical, karyogenetic, and chemotherapeutic studies with parallel cultures of IRC 741 leukemia *in vivo* and *in vitro*. To our knowledge, no previous reports have been made of *in vitro* culture of this leukemia, hence we would like to summarize our studies of its growth *in vitro*.

Materials and methods. We obtained IRC 741 leukemia from Dr. Dunning in November 1963 in the form of solid tumor transplants growing in Fischer rats. A suspension of single cells was prepared by mincing solid tumor masses, suspending the particles in Ringer's solution, and screening the fluid. This suspension was used to initiate an ascites form of the tumor. Both the solid and ascites forms of the leukemia have since been carried in our laboratory by weekly transfer in Fischer rats.

In December 1963, a biopsy of a solid tumor was minced, and was treated with 0.5% collagenase for 1 hour at 37°C in a rotary shaker. The resulting single-cell suspension was inoculated into various cell-culture media under a single layer of perforated cellophane in T-15 flasks. Immediate proliferation occurred in a culture grown on medium RPMI #906(9) supplemented with 5% fetal calf serum. The cells did not adhere firmly to the glass, and hence subcultures of the cells in the supernatant fluid were established in spinner cultures.

Almost all subsequent studies have utilized suspension cultures. A detailed description of the methods used has been published elsewhere(9). Cell counts and viability determinations were made by direct observation of cells exposed to 0.1% trypan blue and placed in Speirs-Levi counting chambers. Cell counts and glucose levels were determined daily. When necessary, adjustment of the pH to 7.2-7.4 was done daily with $NaHCO_3$ or NaOH. Preparations for chromosome studies were made according to methods that we have previously used for cultured cells(10). Cells grown both *in vivo* and *in vitro* have been stored in liquid nitrogen for extended periods, with good viability after recovery. They are usually frozen in cell-culture medium supplemented with 20% fetal calf serum and con-

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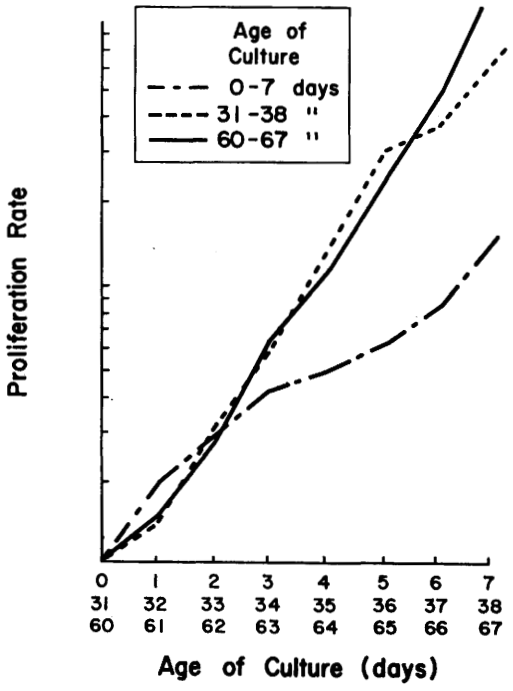

FIG. 1

taining 10% dimethyl sulfoxide.

Results. IRC 741 leukemia was established in culture as strain RPMI #3747 in a spinner flask on December 21, 1963, using medium RPMI #906 supplemented with 5% fetal calf serum. A rapid growth rate was obtained, with a viability of more than 95%, on the basis of trypan blue exclusion. The cell line has since been carried in both short- and long-term suspension cultures (up to 150 days) in volumes from 50 to 14000 ml, and has repeatedly been subcultured for various purposes, such as nutritional assays. Doubling times have varied between 16 to 20 hours, depending on culture conditions. A representative example of the growth rates of a long-term culture is provided in Fig. 1, where the rates for days 0 to 7, 31 to 38, and 60 to 67 are shown. There is some variation in the initial growth rate; but later rates, although higher, remain constant.

Optimal cell concentrations for most rapid logarithmic multiplication with our present media have been 2 to 5 $\times$ 10^5 cells per ml of medium. Supplementation of the medium with 2 to 5% fetal calf serum was found necessary for growth of the cells, 5% being optimal. With less than 2%, growth became slow and erratic; without serum supplementation, growth ceased and viability dropped rapidly. The cells grow quite well in several different media, but consistently best in RPMI #906 and subsequent modifications. This cell line has a very high requirement for folic acid *in vitro,* approximately 1100 mμM folic acid being necessary for half-maximal growth (Fig. 2).

The average survival times for animals routinely carrying IRC 741 leukemia are 8 days with the ascites form and 12 days with the solid form. As few as 10^3 cells injected i.p. will kill the animals, but the survival time is then 17 to 20 days. Cells have been injected into animals after 1 month in culture. Doses as low as 10^4 cells per animal were lethal in 18 to 23 days; but doses of 10^6 cells i.p., in 12 days.

The chromosome constitution of IRC 741 leukemia was determined by Dr. A. A. Sandberg. Cells from ascites show a relatively sharp mode of 42, and range from 38 to 44; cultured cells exhibit a mode of 41, and range from 39 to 82. The chromosome constitution of the cells is unaltered after one and a half years in culture.

Discussion. During the past year and a half, we have established 3 animal leukemia cell lines in culture: L1210, the Shay chloroleukemia, and IRC 741. In contrast to human leukemia cells, which are only seldom established in long-term cultures *in vitro,* animal leukemia cells have requirements that our present media and culture conditions seem to satisfy quite well.

Particularly interesting is the high require-

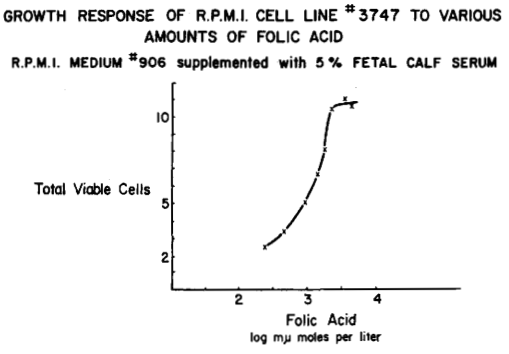

FIG. 2

ment of IRC 741 cells for folic acid. This is comparable to folic acid requirements found in other animal leukemias(12); in this laboratory, L1210 leukemia and the Shay chloroleukemia were found to require approximately 1250 and 830 mμM folic acid, respectively.

IRC 741 cell cultures should be useful for metabolic and chemotherapeutic studies, especially since irradiation, chemicals, and culture conditions provide possibilities for creating variants of the basic cell line. The high degree of stability of the cell line in relation to such phenomena as chromosome numbers, metabolic markers, the high requirement for folic acid, and pathogenicity for rats may prove to be a valuable characteristic of IRC 741 *in vitro*.

Summary. Long-term *in vitro* culture of IRC 741 rat leukemia has been accomplished. During one and a half years in culture, the cell line has remained notably stable in relation to chromosome constitution, high folic acid requirement, growth kinetics, and rat pathogenicity.

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Effect of Propranolol on the Peripheral Circulation.* (30576)

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A newly synthesized compound, propranolol, was found to be a very potent, beta-receptor adrenergic blocking agent(1-3). Since the drug blocks the vasodilator (beta-receptor stimulating) effect of catecholamines without affecting the vasoconstrictor (alpha-receptor stimulating) property, it may be expected that propranolol would not cause vasodilatation of the peripheral vessels. However, Prichard and Gillam(4,5) recently found that propranolol lowered arterial blood pressure significantly in hypertensive patients. The present study was undertaken to investigate the effect of propranolol on the peripheral vascular bed using a technique in which the perfusion blood flow rate was kept constant in anesthetized dogs.

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Methods. Dogs weighing between 20.5 and 26.5 kg were anesthetized with the intravenous injection of sodium pentobarbital (30 mg/kg). In open chest dogs, a femoral artery was perfused with arterial blood at a constant and known rate by means of a Sigma-motor pump as described previously(6). Mean systemic arterial and femoral arterial perfusion pressures were measured continuously with Statham pressure transducers (P23AA). In this set-up, the effect of the drug on the peripheral vascular resistance was evaluated readily by the changes in the perfusion pressure. Heart rate and myocardial contractile force were measured continuously with an Electronics for Medicine (EFM) tachometer and with a Walton-Brodie strain gauge arch(7,8) which was sutured to the right ventricular muscle. All parameters ex-