

Kinetics of Canine Hematopoietic Colonies Grown *in Vitro*¹ (36787)

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(Introduced by D. R. Boggs)

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In vitro systems have been developed for the cloning of hematopoietic cells from a number of mammalian species including the human (1, 2) and mouse (3, 4) in soft agar or methyl cellulose. Among the applications of these techniques have been attempts to identify and quantitate hematopoietic progenitor cells and to assay various substances which may be involved in the regulation of granulopoiesis through their influence on the proliferation of granulocytic colonies. Because of the similarity of human and canine granulocyte kinetics *in vivo* (5) and the need for an animal system suitable for serial blood sampling, the dog was selected as a model system for an investigation into humoral mechanisms in the regulation of granulopoiesis. The purpose of the current investigation was to define the kinetic characteristics of a canine marrow culture system (6, 7) as a background which might be utilized in the assay of possible granulopoietic factors. A detailed description of this system and its characteristics has been presented elsewhere (7).

Materials and Methods. Marrow aspirated in 5–10 ml aliquots from the humeral heads of pentobarbital-anesthetized dogs was collected in equal volumes of isologous heparinized (30–60 units/ml) plasma. After sedimentation of the red cells at a 45° angle for 1–3 hr, the supernatant was removed and

combined with the basic medium so as to yield a final plating concentration of 2 or 3 × 10⁵ cells/ml. Medium 199 in 0.3% agar was supplemented with 1 mM sodium pyruvate, 40 μg/ml L-asparagine, and 75 μg/ml DEAE-dextran. Ten percent fetal calf serum (FCS) was used in some experiments; normal dog and human serum additives were used in a volume of 0.15 ml before plating 1 ml of the agar–marrow suspension. The cultures, in plastic 35 × 10 mm petri dishes, were incubated in 5% CO₂ at 37° in a water-saturated atmosphere. Seven groups of 10 plates were removed at various intervals (days 2–4 to 16–18) and colonies (aggregates of 15 or more cells) were enumerated with a dissecting microscope at 25×. Colony size was determined by counting the cells from 2 to 4 random colonies/plate, smeared, and stained with 0.4% aceto-orcein. At the same time cells were scored by their nuclear morphology as either “mononuclears” or “bands and segmented neutrophils” (Fig. 1). The procedure was carried out in the presence of four different serum “environments”: 10% FCS alone, 10% FCS with 0.15 ml/plate dog serum (DS) additive, 0.15 ml/plate DS, and 0.15 ml/plate human serum (HS). In the latter 2 experiments (without FCS), the largest colonies and a random colony were picked from each plate. Only the random colony data are presented for colony size but the morphologic data from both large and random colonies have been combined since they did not differ significantly.

Results. The results are summarized in Fig. 2 in which the kinetic curves for each colony parameter and serum environment are shown. The curves for colony number and size were normalized to facilitate comparison of different experiments and are constructed

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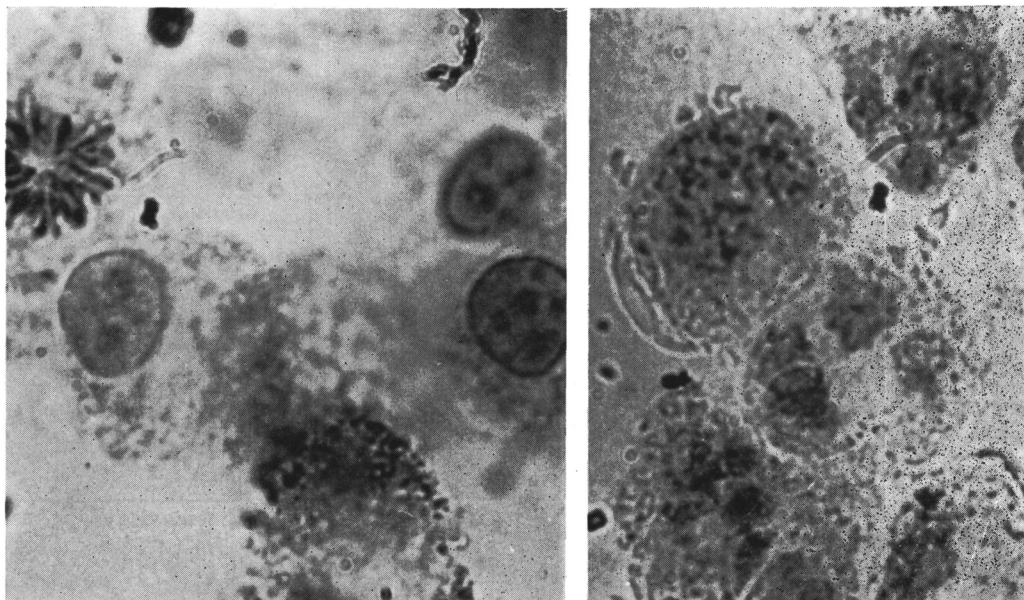


FIG. 1. Colony cell morphology stained with aceto-orcein. Left panel shows typical mononuclear cells with nucleoli and mitosis. Right panel shows typical bands and segmented neutrophils with cytoplasm containing phagocytized granular agar ($\times 1000$).

from values representing a percentage of each respective maximum. The absolute maximum values, the day each maximum was observed and the mean number of values making up each data point on the curves are presented in Table I.

Colony number in all four experiments rapidly rose to a maximum and then declined. The attainment of maximum colony number was somewhat delayed with HS (14 days) compared to DS, DS and FCS, and

FCS alone (7-9 days). A more rapid rate of decline was noted with DS than FCS or HS alone. Colony size kinetics were quite variable: a rise to maximum values was observed with DS (9 days), HS (11 days) and DS and FCS (7 days). A steady decline in mean colony size was observed with FCS alone after day 5.

The kinetics of development of identifiable band and segmented forms (presumably neutrophils) were quite different in the vari-

TABLE I. Absolute Maxima of Colony Parameters (Values/Day).^a

Serum	Inoculum size (cells/plate)	Colony no.	Colony size	Bands and segmented neutrophils (%)
FCS	3×10^5	70/9 (9.6)	92/5 (39.3)	10/7, 12 (37.8)
DS and FCS	2×10^5	65/7 (10.7)	122/7 (19.1)	35/11 (17.6)
DS	3×10^5	39/9 (8.5)	94/9 (7.8)	81/7 (15.3)
HS	3×10^5	60/14 (9.1)	306/11 (8.8)	82/7 (16)

^a Numbers in parentheses are the mean number of samples for each data point on the curves shown in Fig. 2.

ous experiments. No more than 10% bands and segmented forms were found with FCS alone as contrasted to 35% for DS and FCS, and 81-82% for DS or HS alone. The percentage of bands and segmented forms dropped sharply during the later stages of incubation

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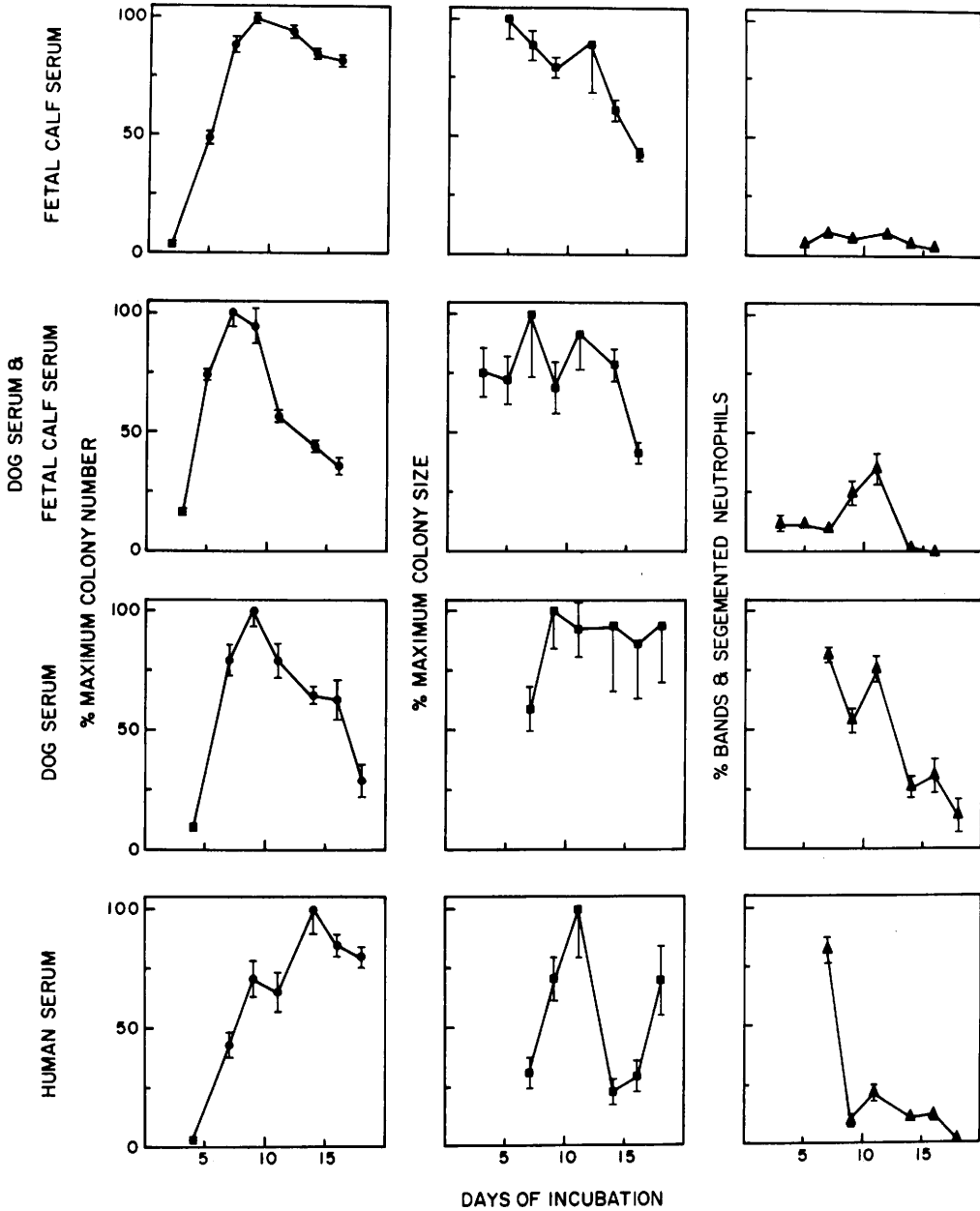


FIG. 2. Dog leukocyte colony kinetics with various serum additives. Three colony parameters: colony number, colony size and cell morphology (bands and segmented neutrophils) are illustrated as they developed chronologically in the presence of the 4 different serum additives. Values for mean \pm standard error are shown. Very small standard errors, which would be hidden by the symbols, are not shown.

as they were replaced by mononuclear cells.

Discussion. Colony number in either FCS, DS and FCS or DS alone was maximal at days 7-9 suggesting this period as the appropriate time for assessing the colony-stimulating activity of dog sera. The development of dog hematopoietic colonies with regard to both size and number was delayed in the presence of human serum. This is consistent with the slower development of human hematopoietic colonies demonstrated by others (1, 2) and suggests that serum factors may be involved in this phenomenon. The dog marrow culture system may be useful in assaying human material. Unlike the serum of adult dogs or humans, FCS may lack a stimulator or contain an inhibitor for the differentiation of canine granulocytic cells *in vitro*. FCS does not, however, inhibit dog marrow colony formation in high concentration, in contrast to dog and human serum (8). The explanation for the significant increase in random colony size on day 18 with human serum is not apparent; a secondary stimulus for proliferation is possible, perhaps due to a persistence of colony stimulating activity in this serum specimen and/or increased sensitivity of mononuclear cells at this time. Further studies would be needed to confirm this.

A kinetic analysis of mouse bone marrow colonies during the first 10 days of incubation has been carried out by Metcalf, Bradley and Robinson (9). Colonies grown in FCS on a kidney feeder layer remained predominantly granulocytic but if leukemic mouse serum, or human serum obtained from patients with certain illnesses (10) was substituted, the colonies lost their granulocytic nature, becoming almost completely mononuclear. A similar sequence was also observed with rat bone marrow colonies in the presence of rat and bovine serum and FCS (11). Normal human serum is inactive in promoting colony development from rodent marrow.

The growth rate of colonies, as measured by number and size, and content of specific cell types is profoundly influenced therefore, in all systems studied by the serum environments, *i.e.*, the source and concentration of

colony stimulating factors (12). Kinetic data may be useful in the design of experiments to evaluate humoral factors which participate in the regulation of granulopoiesis.

Summary. Leukocyte colonies derived from canine bone marrow show different rates of growth and cytological differentiation when grown in the presence of different serum environments. Minimal formation of mature granulocytes was observed with fetal calf serum (FCS) alone compared to that observed with dog or human serum. The growth kinetics and composition of canine leukocyte colonies are dependent upon the source of colony stimulating activity used. This system may be useful in assessing granulopoietic factors in canine and human serum.

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