Ascorbic Acid: A Culture Requirement for Colony Formation by Mouse Plasmacytoma Cells

Abstract. *Plasmacytoma stem cells explanted from mice formed colonies in culture only in the presence of* L-*ascorbic acid; this vitamin was not needed for the formation of granulocytic colonies. Isomers of* L-*ascorbic acid with less antiscorbutic activity also promoted plasmacytoma colony formation, but less effectively. Other redox compounds without antiscorbutic activity and an antioxidant were ineffective.*

The precursors of normal granulocytes and the stem cells of transplantable mouse plasmacytomas both form colonies in culture when comparable methods are used (*1*). The culture requirements for plasmacytoma cells, however, are more stringent than those for normal marrow. Plasmacytoma cells form colonies when the cultures are fed daily with freshly prepared tissue culture medium or medium stored at −20°C, but not with medium stored at 4°C. This finding was interpreted as indicating a requirement for a labile factor, since mixing experiments failed to demonstrate inhibition of growth by inactive culture medium (*1*). In contrast, normal granulopoietic progenitors do not appear to require a labile factor, since these will form colonies in medium stored for months at 4°C and without refeeding (*1*). In this report we present evidence that the labile growth factor required for plasmacytoma cells is L-ascorbic acid, and that its role in culture is correlated with its antiscorbutic action.

The cell culture system for mouse plasmacytoma stem cells (*1*) is a modification of that devised by Pluznik and Sachs (*2*) and Bradley and Metcalf (*3*) for normal mouse marrow. The cultures consist of two layers in 35-mm plastic petri dishes: the bottom layer contains renal tubules immobilized in 0.5 percent agar as described by Abrahams *et al.* (*4*). The top layer of 0.3 percent agar contains an appropriate number of cells from spleens of BALB/c mice infiltrated with Adj. PC-5 plasmacytoma cells (*5*) and 10^6 heavily irradiated (2000 rad) cells of the same origin. Both layers have α medium (*6*) supplemented with 10 percent pooled human serum (*7*). To permit the daily refeeding required for plasmacytoma cells, six holes are placed symmetrically in the bottom of the dish. Daily, 0.5 to 1.0 ml of freshly prepared α medium with human serum is added to the dishes. Cultures are incubated in a humidified atmosphere continuously flushed with 7 percent CO_2. Colonies are scored after 6 or 7 days of growth, by using an inverted microscope; colonies derived from plasmacytoma cells are easily distinguished from normal granulocytic colonies by morphology (*1*).

The labile factor in active α medium was found to be L-ascorbic acid by testing the components of the medium in sets. Table 1 contains the results of a representative experiment demonstrating the specificity of the requirement for ascorbic acid by plasmacytoma cells. The α medium that was stored at 4°C failed to support colony formation by plasmacytoma cells; freshly thawed medium was active, and the inactive α medium became active when supplemented with L-ascorbic acid (*8*). In contrast, normal marrow yielded colonies in comparable numbers in all three groups. The ability of freshly prepared α medium to support the growth of plasmacytoma cells was compared with the same medium lacking L-ascorbic acid, prepared at the same time. The plasmacytoma cells did not form any colonies in the cultures prepared with the latter medium, but growth was normal with the former. Thus, L-ascorbic acid is essential for the formation of colonies by plasmacytoma cells, but not for normal marrow.

We tested two isomers of L-ascorbic acid for capacity to support colony formation by plasmacytoma cells. Isoascorbic acid has approximately 1/20 of the antiscorbutic activity of L-ascorbic acid (*9*, *10*); D-ascorbic acid is also less effective than L-ascorbic acid in guinea pigs (*9*, *11*), although in both instances decreased potency may in part be attributable to a shortened in vivo half-life of the compounds (*12*). Figure 1 illustrates the results of a representative experiment in which the three isomers were tested in culture at concentrations ranging from 0.03 to 3.0 m*M*.

Table 1. Specific requirement of L-ascorbic acid for colony formation by plasmacytoma cells in culture. Cells from plasmacytoma-infiltrated spleen (3×10^4 per dish) and normal BALB/c bone marrow (5×10^5 per dish) were plated in parallel. The plasmacytoma and granulocytic colonies were counted on day 6 or 7. All cultures were fed daily with one of: α medium stored for 1 month at −20°C, 4°C, or the latter supplemented just before use with L-ascorbic acid at a concentration of 0.3 m*M*. L-Ascorbic acid was initially dissolved in distilled water at 100 times the desired concentration and added to the medium in 1 volume per 100. Each set had five dishes.

Storage of medium used for feeding	Number (mean ± S.E.) of	
	Plasmacytoma colonies per 3×10^4 cells	Granulocytic colonies per 5×10^5 cells
1 month at −20°C	299 ± 18	90 ± 9.5
1 month at 4°C	0	88 ± 9.5
1 month at 4°C with added L-ascorbic acid	279 ± 21	79 ± 8.9

Table 2. Compounds ineffective in promoting colony formation by plasmacytoma cells in the absence of L-ascorbic acid. These compounds were tested at 0.1, 0.3, 1.0, and 3.0 m*M*, as described in Fig. 1, except that α-tocopherol and 3-methylcholanthrene were dissolved initially in ethanol and xylene, respectively. For the toxicity tests varying concentrations of these compounds were added with 0.3 m*M* of L-ascorbic acid.

Category	Compound	Redox potential $E^{o\prime}$ (volt)*	Effect on growth when added with L-ascorbic acid
Redox system	Hydroquinone	− 0.34	Toxic
	Dichlorophenolindophenol	0.217	Toxic
	Methylene blue	0.011	Toxic
	Glutathione	−0.23, −0.34	Enhancing
	Cysteine	−0.34	Enhancing
Antioxidant	α-Tocopherol		No effect
L-Ascorbic acid precursors	L-Gulonolactone		No effect
	D-Glucuronic acid		No effect
Miscellaneous	3-Methylcholanthrene		No effect
	Folinic acid		No effect

* Measured at *p*H 7 and temperature between 20° and 30°C, redox potential for L-ascorbic acid is 0.508 (*23*).

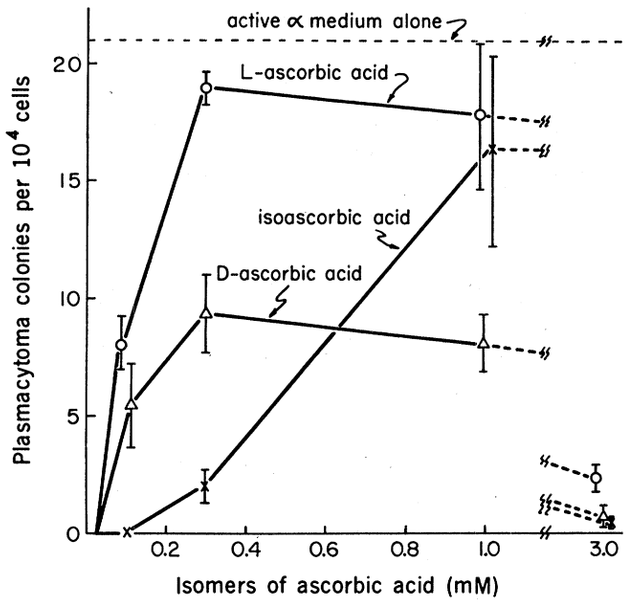

Fig. 1. Effects of ascorbic acid isomers on the colony formation by plasmacytoma cells. Plated in each dish were 10^4 cells from plasmacytoma-infiltrated spleen. The cultures were fed daily with inactive α medium (stored for 1 to 2 months at 4°C) supplemented with one of three isomers at various concentrations as shown. Each isomer was prepared and added as described in Table 1. Each point and vertical bar represent mean and standard error of four dishes. The number of colonies obtained with active α medium (freshly thawed) is shown by a dotted line.

The L-ascorbic acid was the most effective additive; full activity was achieved at 0.3 m*M*. In contrast, 1.0 m*M* isoascorbic acid (*13*) was required for comparable efficiencies of colony formation, while D-ascorbic acid (*14*) was less effective at all levels tested. For all three compounds, colony-forming efficiency decreased when 3.0 m*M* was added to the medium. The data of Fig. 1 are consistent with a correlation between antiscorbutic activity in vivo and capacity to promote plasmacytoma colony formation in culture.

The compounds listed in Table 2 were also tested in the culture system because they are members of classes that might have activities similar to L-ascorbic acid. For each compound, concentrations between 0.1 and 3.0 m*M* were ineffective in restoring activity to inactive α medium. In each experiment, two positive controls, one consisting of freshly thawed α medium and the other of inactive α medium with added L-ascorbic acid, yielded the expected efficiency of plasmacytoma colony formation. The compounds were also tested for toxicity by adding them with L-ascorbic acid to inactive α medium. The redox compounds hydroquinone (*15*), 2,6-dichlorophenolindophenol (*15*), and methylene blue (*15*) were not only inactive but also proved toxic when added with L-ascorbic acid. In contrast, glutathione (*8*) and cysteine (*8*), though inactive by themselves, doubled the efficiency of colony formation in the presence of L-ascorbic acid. It is not known whether these effects result from a direct action of the compounds on the cells or through effects on L-ascorbic acid related to the redox potential of the compounds.

The remaining compounds were neither effective by themselves nor able to change colony-forming efficiency when added with L-ascorbic acid. α-Tocopherol (*8*) was tested because it is antioxidant. L-Gulonolactone (*16*) and D-glucuronic acid (*16*) are metabolic precursors of L-ascorbic acid. 3-Methylcholanthrene (*13*) was tested because it has been reported to have slight antiscorbutic activity (*17*), and folinic acid (*8*) since L-ascorbic acid has been implicated in the conversion of folic acid to folinic acid (*18*). The negative results obtained with the compounds of Table 2 lend support to the view that the action of L-ascorbic acid is specific.

The data of this report were obtained using Adj. PC-5; however, the addition of L-ascorbic acid to inactive α medium restored its capacity to support colony formation by two other plasmacytomas, MOPC 104E and MOPC 46B (*5*). Thus, at least these three lines of mouse plasmacytomas, but not normal mouse granulopoietic precursors, require L-ascorbic acid for growth in culture. Normal mouse granulopoietic precursors were used in the comparison because they are closely related to hemopoietic stem cells (*19*); these are required for regeneration of the hemopoietic system after injury and are an important dose-limiting factor in the chemotherapy of multiple myeloma. A special requirement for ascorbic acid has not been reported for the function of normal immunocompetent cells in culture; indeed, normal mouse spleen cells mixed with sheep erythrocytes can give rise to hemolysin-forming cells in culture, even when incubated in medium stored for months at 4°C. The addition of L-ascorbic acid in the amount necessary for optimal growth of plasmacytoma cells to such medium does not alter the hemolysin-forming cell response (*20*).

Our data do not permit any conclusion about the comparative nutritional requirements of normal marrow and plasmacytoma cells in vivo. However, one instance exists where cells of similar embryological origin require L-ascorbic acid both in culture and in vivo. Odontoblasts regress to cuboidal or squamous form in scorbutic guinea pigs (*17*) and chick chondrocytes disintegrate unless cultivated in medium supplemented with L-ascorbic acid (*21*). The model of plasmacytoma in the mouse is not appropriate to study this aspect of the problem, since mice do not become scorbutic (*22*); suitable plasmacytoma models are not available in the guinea pig. Perhaps the critical studies must be made in man, a species susceptible to both scurvy and multiple myeloma.

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References and Notes

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5. Adj. PC-5, in the 97th transplant generation, was obtained in ascitic form from Dr. Michael Potter, National Institutes of Health. In our laboratory, it was adapted for growth in the spleen and has been transplanted fortnightly by the intravenous injection of 10^6 to 10^7 tumor cells. The present experiments were carried out with cells from intravenous transplant generations 107 to 111. Dr. Potter also supplied us with additional plasmacytoma lines, MOPC 104E and MOPC 46B, which had been carried continuously by subcutaneous passage. These two tumors are maintained at our laboratory by subcutaneous passage. Plasmacytoma cell suspensions were prepared from the spleens or subcutaneous tumors of mice given previous injections of plasmacytoma cells by mincing with scissors and washing through a fine wire mesh screen with culture medium.
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The Brain as a Parallel Coherent Detector

Abstract. *Knowledge of the bioelectric signal-to-noise ratios in rat brain makes it possible to demonstrate for the first time that the brain functions as a coherent signal detector, an important class of detectors that are explicitly formulated within the statistical theory of communication. Within an afferent neuronal channel of a single modality, the brain functions as a parallel signal processor.*

In the visual system in the rat during photic stimulation the relative bioelectric signal-to-noise power ratio (S^2/N^2) for input at the contralateral eye as compared to the ipsilateral eye measured at either the dorsal lateral geniculate nucleus (LG) or at the visual cortex (VC) corresponds to the known decussation ratio of neurons in the optic nerve (*1*). A narrow bandpass measuring technique was used to define a signal channel (*1*). Signal-to-noise ratios were computed by subtracting the integrated filtered brain output obtained during a no-input condition (N) from the output obtained during a matched period of channel-tuned photic stimulation (S') and dividing the obtained value (S) by the output without input (N). In this procedure, the output without input is defined as background noise. Thus, signal-to-noise ratio $= (S' - N)/N = N$.

In accordance with empirical findings

$$(S^2/N^2)_1/(S^2/N^2)_2 = \psi_1/\psi_2 \qquad (1)$$

where $(S^2/N^2)_1$ is the signal-to-noise power ratio at a given brain station (say, LG) when the signal is transmitted through a communications channel 1 (C_1), $(S^2/N^2)_2$ is the signal-to-noise power ratio at the given brain station (LG) when the signal is transmitted through a communications channel 2 (C_2), and ψ_1/ψ_2 is the ratio of the number of visual afferent neurons carrying a photic signal in channel C_1 compared to the number of neurons carrying the signal in channel C_2.

This signal processing property of the brain may now be formally compared with various signal detector schemes that are well known in statistical communications theory. The *coherent detector* is of particular importance in that it is theoretically the most efficient of the stochastic signal detection systems (*2*).

If, in a communications system, S/N is measured on a single sample during time unit τ (for example, seconds), and if upon integration over repeated samples T (τ) then

$$S/N = (aT)^{1/2}\,(\dot{S}/\dot{N}) \qquad (2)$$

where a is a scale coefficient and $(\dot{S}/\dot{N})$ is (S/N) for the unit sample. Then it can be said that the system is functioning as a coherent signal detector (*2*). Other kinds of stochastic signal detectors will also yield an increase in signal-to-noise ratio as a function of T, but will do so with less efficiency; for example, for the square law detector, $S/N \approx (aT)^{1/4}\,\dot{S}/\dot{N}$. If $(S/N)_1$ is the signal-to-noise ratio measured over time period T_1, and if $(S/N)_2$ is the signal-to-noise ratio measured over time period T_2—where $(S/N)_1 = (\dot{S}/\dot{N})_2$, that is, S/N for the unit sample is the same for T_1 and T_2—and if the scale coefficient a is constant, then from Eq. 2

$$(S/N)_1/(S/N)_2 = (aT_1)^{1/2}\,(\dot{S}/\dot{N})/(aT_2)^{1/2}\,(\dot{S}/\dot{N})$$

$$(T_1)^{1/2}/T_2^{1/2} = (S/N)_1/(S/N)_2$$

$$T_1/T_2 = (S^2/N^2)_1/(S^2/N^2)_2 \qquad (3)$$

Equation 3 states that for any given coherent detection system the ratio of sample time periods (T) is equal to the ratio of their respective signal-to-noise power ratios (S^2/N^2). Then any system satisfying Eq. 3 can be said to function as a coherent signal detector for T_1 and T_2.

Combining Eqs. 1 and 3

$$T_1/T_2 = (S^2/N^2)_1/(S^2/N^2)_2 = \psi_1/\psi_2 \qquad (4)$$

On the basis of Eq. 4, we conclude that for photic stimulation within the context of our experimental paradigm (at least), the brain functions as a coherent signal detector (*3*).

On the basis of Eq. 4 by implication

$$\psi_1/\psi_2 \equiv T_1/T_2 \qquad (5)$$

Since ψ refers to the number of neurons carrying a signal in a communications channel within the brain, and T refers to the number of repeated samples of a signal integrated over time T (τ), how is the equivalence of the ratios for ψ and T (expression 5) reconcilable? The equivalence of the ψ and T ratios becomes clear once we understand the general statistical concept of the sample space. In the common treatment of signal detection in communications theory, sample space is enlarged by summing repeated samples over time. This corresponds to the usual model of the serial information processor. However, sample space may also be enlarged by parallel processing during a single time unit. (Thus the probability of heads or tails for coins can be established by either tossing one coin n times, or tossing n coins one time.)

My results illustrate the operation of the brain as a parallel signal processor. In the experiments (*1*) underly-