Table 2. Initial velocity of seeds of four species of *Arceuthobium*.

Species	No. of seeds	Velocity* (cm/sec)
A. cyanocarpum	131	2130 ± 30
A. douglasii	125	2230 ± 30
A. cryptopodum	146	2540 ± 40
A. americanum	124	2610 ± 20

* Mean ± standard error.

10^{-6} second) were made every 104 × 10^{-5} second. The length of the flash period for the strobe unit used was approximately 5 × 10^{-3} second, so this resulted in from three to four exposures of a seed in each frame (Fig. 2). The distance traveled in the known time interval was measured and the seed velocity calculated. The field of view of the camera allowed about the first 10 cm of seed flight to be photographed.

Four Colorado dwarfmistletoes were studied: (i) *Arceuthobium americanum* Nutt. ex Engelm., a parasite of *Pinus contorta* Dougl.; (ii) *A. campylopodum* Engelm. f. *cyanocarpum* (A. Nels.) Gill (here abbreviated *"cyanocarpum"*) on *Pinus flexilis* James; (iii) *A. vaginatum* (Willd.) Presl f. *cryptopodum* (Engelm.) Gill (here abbreviated *"cryptopodum"*) on *Pinus ponderosa* Laws.; and (iv) *A. douglasii* Engelm. on *Pseudotsuga menziesii* (Mirb.) Franco. Ten to twenty branches bearing dwarfmistletoe plants with mature fruits were cut, the ends were placed in water, and the branches were transported to the laboratory. Usually, velocity measurements were made on the same day as collection, and only the most mature fruits were used. Measurements were obtained from the 1963 and 1964 seed crops for each dwarfmistletoe.

Size, weight, volume, and specific gravity of seeds of the four dwarfmistletoes studied are given in Table 1. Initial velocity measurements of 526 seeds of the four species are given in Table 2. There were no statistically significant differences between the 1963 and 1964 measurements for any of the four dwarfmistletoes, so data for the 2 years were combined for each species. Velocities ranged from 2100 cm/sec for *A. cyanocarpum* to 2600 cm/sec for *A. americanum*, and averaged 2400 cm/sec for all seeds measured. The difference in velocities between seeds of *cyanocarpum* and of *douglasii* was not significant, nor was that between seeds of *cryptopodum* and of *americanum*. However, the difference between the group with the lower velocity (*cyanocarpum* and *douglasii*, 2200 cm/sec) and the group with the higher velocity (*cryptopodum* and *americanum*, 2600 cm/sec) was significant at the 1 percent level of probability.

The initial velocities are thus considerably greater than the velocity of 1370 cm/sec previously estimated indirectly *(1)*. That the previous estimate was so low can probably be accounted for by the use of a formula in which no allowance was made for the seeds tumbling in flight, and in which airflow around the seeds was assumed to be laminar—that is, the frictional force was assumed to be proportional to the seed velocity. Recent calculations based on the assumption of an equivalent sphere (with the same terminal velocity as mistletoe seeds) showed that the Reynolds number calculated from the initial velocity of the mistletoe seed was well beyond the critical velocity for the transition from laminar to turbulent flow about the seed. The initial resisting force calculated for a turbulent flow was of the order of 15 times that for laminar flow.

As is evident from the data in Tables 1 and 2, there is no direct relationship between seed velocity and seed size. *Arceuthobium douglasii* and *A. americanum* have seeds of the same size, but the former is in the group having a low velocity while the latter is in the group with a high velocity.

The two dwarfmistletoes in the group with high velocity are larger plants (the shoots usually being 7 to 12 cm high) than those in the group with a low velocity (shoots 2 to 5 cm high). Possibly the water regime of the larger mistletoes enables them to build up higher hydrostatic pressures within the fruits, thus resulting in greater seed velocities.

We do not have sufficient data to compare seed velocity with horizontal distance of seed flight for the various dwarfmistletoes. However, the longest horizontal distance of seed flight that we have measured (14.6 m, *2*) is for *A. cryptopodum*, a species in the group having a high velocity.

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Control of Glutamine Synthetase in the Embryonic Retina in vitro

Abstract. *Glutamine synthetase activity in the neural retina of the chick embryo increases sharply during terminal differentiation of this tissue. This characteristic increase can be reproduced in cultures of retinal tissue fragments from late embryos. A similarly sharp increase can be elicited precociously in younger retina by culturing in medium with adult serum. Both the precociously elicited and the later increase in enzyme activity require continuous protein synthesis; both can be suppressed during the first 24 hours of culture by blocking RNA synthesis or by removing the adult serum. Subsequently, the increase in enzyme activity becomes progressively less dependent on RNA synthesis and on the continuous presence of adult serum. This transition is attained more rapidly in the older retina. The data suggest a progressive stabilization of the enzyme-forming system during differentiation.*

Recent work on regulatory mechanisms in microorganisms has stimulated interest in the availability of embryonic systems in which gene function and the appearance of differentiation products might be experimentally manipulated and modified. This report summarizes further features of a system previously shown *(1, 2)* to have certain advantages for such work: control of glutamine synthetase *(3)* in the neural retina of the embryonic chick in vitro and in vivo in relation to the differentiation of this tissue.

Rudnick and Waelsch *(4)* found that glutamine synthetase (GS) activity in the neural retina of chick embryos is at low levels until about the 17th day of incubation, after which time the activity increases very rapidly until af-

ter hatching (*1, 4*). This pattern of GS development is characteristic of and unique to the neural retina and parallels closely the morphological and functional differentiation of this tissue (*1, 4, 5*); the pattern is not complicated by mitotic activity, since cell proliferation is minimal in the retina after the 10th day of embryonic development (*6*).

A similarly rapid rate of increase in GS activity can be obtained in vitro in cultures of retina tissue from 16- or 17-day-old embryos (*1, 2*). It is also possible to elicit an increase in activity of this enzyme days in advance of the normal schedule of differentiation by explanting retinas from much younger embryos into a suitable culture medium (*1, 2*). Thus, when fragments of retina from 10-day embryos are cultured in Tyrode's saline containing horse serum (10 percent) in flasks on a gyratory shaker, GS activity increases at a rate similar to that normally obtained in later development *in situ* (*1, 2*). Like the normal increase, this precocious rise in GS activity in culture is characteristic of the neural retina and is accompanied by cytodifferentiation (*5*) but not by a noticeable increase in cell proliferation.

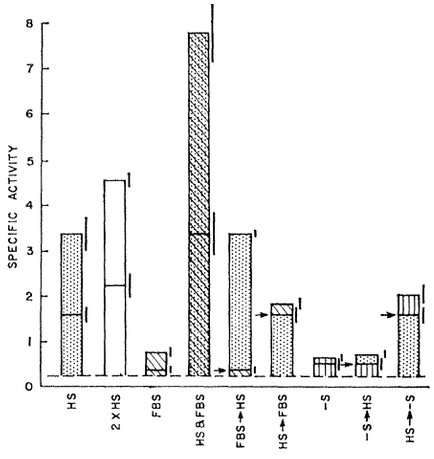

Fig. 1. Glutamine synthetase in neural retinas of 10-day embryonic chicks cultured in mediums of different serum content. Lower segment of each bar: mean specific activity attained during 1st day in culture. Upper segment of each bar: mean specific activity attained during 2nd day in culture. Vertical lines: standard deviation of data represented by adjacent bar segments. *HS*, Culture medium containing 10 percent horse serum; *2 × HS*, medium containing 20 percent horse serum; *FBS*, medium containing 10 percent fetal bovine serum; *—S*, medium lacking serum. Arrow indicates transfer of cultures from first medium to second after one day in culture. Dashed baseline represents enzyme activity at time of explantation.

That protein synthesis is required for the increase in retinal GS activity was demonstrated in cultures of retinal tissue by using inhibitors of protein and RNA synthesis (*2*). At concentrations inhibitory to amino acid incorporation, puromycin blocks, reversibly, any further increase in GS activity regardless of the age of the retina at explantation, the duration of cultivation, or the enzyme activities attained before the addition of puromycin. Actinomycin D at a concentration inhibitory to RNA synthesis blocks the increase in GS activity in retinas from both 10- and 16-day embryos, if added at the time of explantation; thus, the elicitation of the sharp rise in retinal GS is associated with RNA synthesis. However, when the retina is first cultured in normal medium and the enzymic rise is initiated before exposure of the culture to actinomycin, the subsequent increase in GS activity becomes progressively less dependent on RNA synthesis (*7*). The development of this independence of RNA synthesis is a function of both the duration of precultivation in normal medium, and the age of the retina: in the 16-day retina, this independence develops within 24 hours; in the 10-day retina, after 48 hours. Thus the increase in GS activity in the cultured retina requires continuous protein synthesis and is at first dependent on and later independent of RNA synthesis; this transition suggests that, as retinal development progresses, a stable GS-forming system accumulates and cytoplasmic control mechanisms play an increasing role in regulating GS activity.

Elucidation of factors resulting in precocious activation of the GS-forming system in cultured retina could provide clues concerning mechanisms controlling the normal development of this enzyme and add to the system's usefulness in studying the regulation of gene-dependent and independent processes in embryonic cells. The precocious increase in GS activity in the explanted 10-day retina could be due either to isolation of the tissue from inhibitors present in the early embryo or to exposure of the tissue to stimulatory or derepressive factors in the serum-containing culture medium. To examine these alternatives, horse serum was omitted from the medium and the specificity of its effect was tested by

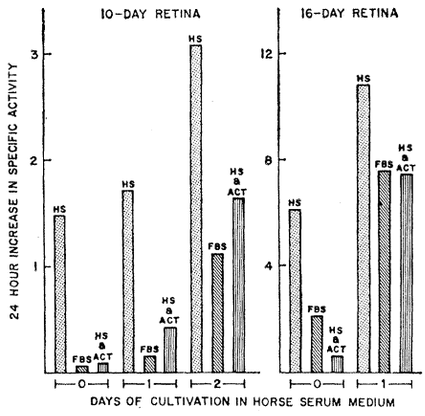

Fig. 2. Effects of omitting horse serum and of adding actinomycin on the increase in glutamine synthetase in cultured retina, as a function of age and period of precultivation in horse-serum medium. Bars represent activity increases during 24 hours after transfer of the tissue from horse-serum medium. *HS + Act*, transferred to 10 percent horse-serum medium containing 10 μg/ml of actinomycin D. Other symbols as in Fig. 1.

substitution of or supplementation with fetal bovine serum and other serums. Additional cultures were transferred from one medium to another after 24 and 48 hours of cultivation. Some representative data obtained with 148 such cultures of 10-day retinas (retinas from two eyes per culture) are summarized in Figs. 1 and 2. These data suggest the following tentative conclusions.

1) In the absence of horse serum, or when it is replaced with *fetal* bovine serum, there is no substantial increase in GS activity in the explanted 10-day retina during 48 hours in culture; therefore, mere isolation of the early retina from the embryonic environment is not the direct cause of the rapid precocious rise in GS activity.

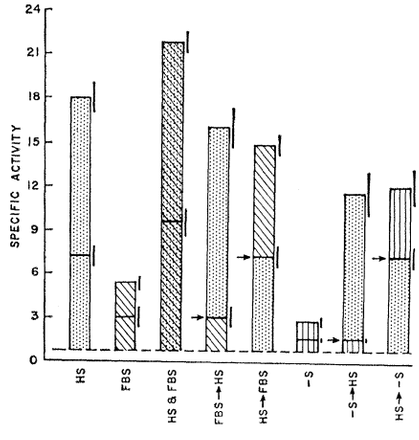

Fig. 3. Glutamine synthetase in cultured neural retina of 16-day embryonic chick. All symbols as in Fig. 1.

2) Although fetal bovine serum does not elicit under these conditions the rapid increase in GS activity, it does not inhibit the process when added to the medium with horse serum. Therefore, the precocious elicitation of the rise in GS activity may be due to a factor present in horse serum but, apparently, low or lacking in fetal serum (*8*). Since other *adult* sera can also elicit the precocious increase in GS activity, the postulated factor appears not to be restricted to adult horse serum (see below).

3) While fetal serum appears to lack an effective concentration of this essential factor, it has a promoting effect on the GS-forming system: when added to the medium with horse serum, fetal serum stimulates increase in GS activity to values significantly higher than additive, and greater than can be obtained by doubling the concentration of horse serum. Furthermore, when 10-day retina is transferred to horse-serum medium following a 24-hour exposure to fetal-serum medium, GS activity increases more rapidly than in retinas maintained continuously in horse-serum medium, as if the fetal serum had "primed" the retina for a response to horse serum. Fetal serum may thus contain a "priming" factor demonstrable by the simultaneous or subsequent exposure of the retina to horse serum.

4) When 10-day retina is cultured for only 24 hours in horse-serum medium and is then transferred to fetal-serum medium or to a serum-free medium, there is only a slight additional increase in GS activity; however, after 48 hours cultivation in horse serum, transfer to fetal bovine serum permits a substantial further increase (Fig. 2) suggesting that, during 48 hours of culture in the horse-serum medium, the GS-forming system in the 10-day retina becomes independent of continuous elicitation by this adult serum.

Data obtained in 86 similar cultures of 16-day retina are summarized in Figs. 2 and 3. Generally, the results are similar to those obtained with 10-day retina, with this exception: after only 24 hours of culture the GS-forming system of the 16-day retina becomes independent of the requirement for horse serum. It is of considerable interest that in both the 10-day and the 16-day retina the development of independence of horse serum parallels the development of insensitivity to actinomycin (Fig. 2). This correspondence may be coincidental or may indicate stabilization of the GS-forming system caused by an effect of horse serum.

Precocious enhancement of the GS-forming system in the 10-day retina in vitro can also be caused by other *adult* serums: chicken, bovine, and human. In this effect, the adult serums may be considered to simulate conditions affecting the retina in the late chick embryo at the time of the normal increase in retinal GS activity. On the other hand, serum and extracts from early chick embryos resemble fetal serum in failing to elicit in vitro the precocious effect, and simulate in this respect the conditions affecting the retina in the early chick embryo prior to the normal rise in GS activity.

As a step toward testing the above assumptions we have isolated an active fraction from horse serum which causes a precocious increase in retinal GS activity in vitro. Its characterization should facilitate further studies on its site and mode of action in "turning on" or stabilizing the GS-forming system in retinal cells, with reference to retinal differentiation.

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7. Concentration of actinomycin D used: 10 μg/ml; RNA synthesis measured by incorporation of uridine-2-C^{14} (D. L. Kirk, in preparation).
8. This activation is similarly observed with dialyzed horse serum; it is not readily explained on the basis of variations in glutamate or glutamine concentrations, although these amino acids have some modifying effects on the amount of enzyme activity in culture (*2*).
9. Supported by NSF research grant G-23852, and by NIH research grant HD 01263-06. The assistance of Nilda Saenz is gratefully acknowledged.

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Granulocytosis-Promoting Extract of Mouse Tumor Tissue: Partial Purification

Abstract. *A granulocytosis-promoting extract of mouse tumor tissue was partially purified by differential precipitation with cold 20- to 30-percent ethanol at pH 6.5, "salting out" with 50- to 75-percent saturated ammonium sulfate, and filtration through Sephadex G-75 dextran gel. In intact mice, single doses of the extract (at least 12 micrograms per gram of body weight) induced up to a 30-fold increase in circulating granulocytes and a two- to threefold decrease in circulating mononuclear cells within 3 to 7 hours of intravenous injection.*

A variety of human and animal tumors have been reported to elaborate humoral substances unrelated to the type of tumor tissue. Adrenocorticotropic hormone (*1*), antidiuretic hormone (*2*), thyroid-stimulating hormone (*3*), a hypoglycemic factor (*4*), a lipid-mobilizing factor (*5*), a parathyroid-stimulating factor (*6*), erythropoietin (*7*), and leukopoietin (*8*) figure among these substances.

Previously, we reported (*9*) that massive granulocytosis associated with extramedullary granulopoiesis and a trend toward reduction of platelet numbers was evoked in CE and (BALB/C × CE)F_1 hybrid mice by a transplantable mouse tumor, CE 1460 mammary cancer, but that no such changes could be induced in BALB/C or (BALB/C × CE)F_1 hybrid mice by another transplantable mouse tumor, BALB/C 2301 mammary cancer. Instead, the latter tumor was associated with normal numbers of leukocytes and with fluctuations of platelet numbers between "baseline" levels (comparable to those found in normal mice) and elevated levels (up to two and one half times above the "baseline"). Our present investigation was based on the hypothesis that the CE 1460 tumor tissue is the source of a granulocytosis-promoting factor (GPF) which induced the granulocytosis and perhaps also the extramedullary granulopoiesis observed in mice bearing CE 1460 tumors.

Fresh-frozen CE 1460 tumor tissue was homogenized with cold 0.4*N* perchloroacetic acid in a Vir-Tis homogenizer. The acid-insoluble residue was washed successively with 75-percent, 95-percent, and 100-percent ethanol. The residue was then boiled for 60 minutes in 15 volumes of 10-percent