acid and glutamine are known as products of cerebral metabolism(10), but it can be suggested that in the case of uremia the greater part of free amino acids comes from the blood, because there is a parallel increase in the blood *and* c.s.f. Positive confirmation of this hypothesis may be obtained by injection of labelled amino acids in experimental uremia and detection of their increase in the c.s.f.

Summary. Free α-amino-nitrogen and free amino acids have been measured in the serum, urine, and cerebrospinal fluid (c.s.f.) of 51 patients with severe uremia and 20 patients with renal disease without uremia. The results have been compared with those obtained from a study of 50 patients without renal disease. Severe uremia was found to be associated with a highly significant increase in the levels of almost all amino acids in the cerebrospinal fluid. In particular aromatic amino acids and glutamine are elevated. This increase probably reflects increased blood levels of free amino acids and an increase in the permeability of the blood-brain barrier in uremia.

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Behavior on Transfer of Serum Stimulated Bone Marrow Colonies.* (32563)

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Sera from mice with spontaneous or viral induced leukemia stimulate certain mouse bone marrow cells to proliferate and form cell colonies in semi-solid agar cultures(1,2). Most normal mouse sera exhibit weak or no colony stimulating activity. Developing colonies are initially granulocytic but after 6 days become composed almost entirely of mononuclear cells(3). The present studies have examined the proliferative capacity of developing bone marrow colonies after transfer to new agar plates and the dependence of the colony cells on active serum for continued multiplication.

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Materials and methods. Mice. Three-month-old male DBA/1 mice were donors for bone marrow cells. *Sera.* Sera were obtained from Swiss ICR/Ha mice aged 5-8 months with advanced lymphoid leukemia, induced by neonatal infection with a leukemia-inducing virus whose isolation and properties have been described elsewhere(4,5). Normal Swiss sera were from mice of the same age. AKR sera were from 6-8-months-old mice with lymphoid leukemia. Sera from BALB/c mice with lymphoid leukemia induced by the Moloney leukemia virus were supplied by Dr. J. Moloney, National Cancer Institute, Bethesda. Normal human sera were obtained from the Institute blood bank and sera from patients of similar ages with mononucleosis were supplied by Dr. Britta Wahren, Karolinska Institutet, Stockholm.

Bone marrow culture technique. The technique for induction of bone marrow colony

formation has been described in detail elsewhere(1,2). 50,000 DBA/1 bone marrow cells were cultured in 35 mm Petri dishes containing 1 ml of 0.3% agar containing Eagle's medium, 10% fetal calf serum and 10% trypticase soy broth. Plates were incubated at 37°C in 5% CO_2 in air. Colony counts were performed using a dissecting microscope.

Transfer technique. Using a fine Pasteur pipette, individual colonies were removed from the culture plate with a small volume of the surrounding agar. Colonies were placed back on the surface of the donor plate, checked to ensure that no extraneous cells had been included in the agar droplet, and a count made of the cells in the colony. Colonies were then transferred to new plates containing 1 ml of 0.3% agar in culture fluid, with or without added test serum. In most cases the colonies became attached to the surface of the agar in the transfer plate and were surrounded by the small volume of agar (approximately 4 × the volume of the colony itself) removed from the donor plates. For transfer of dispersed colony cells, colonies were transferred to tubes containing 1 ml of liquid 0.3% agar in culture fluid held at 37°C. Colony cells were dispersed by vigorous aspirations up and down, transferred to Petri dishes and the medium allowed to gel. These dishes were examined under the dissecting microscope to ensure that no cell clumps were present and then incubated.

Colony cell counts. Colonies were removed using a fine Pasteur pipette and stained on slides with 0.4% orcein in 60% acetic acid. Cell counts were performed at ×200 and cytological classification at ×1000.

Results. Growth of intact colonies on transfer. Colony formation by DBA/1 bone marrow cells was stimulated by the addition of 0.05 ml of leukemic Swiss serum to each culture plate. Colonies achieved maximum size by day 11 of incubation, then colony cell numbers decreased (Fig. 1). Intact colonies were removed after 4 or 7 days of incubation and placed on new plates containing 0.05 ml of the same leukemic serum. Colony cell counts after 7 days of further incubation showed that transferred colonies achieved a

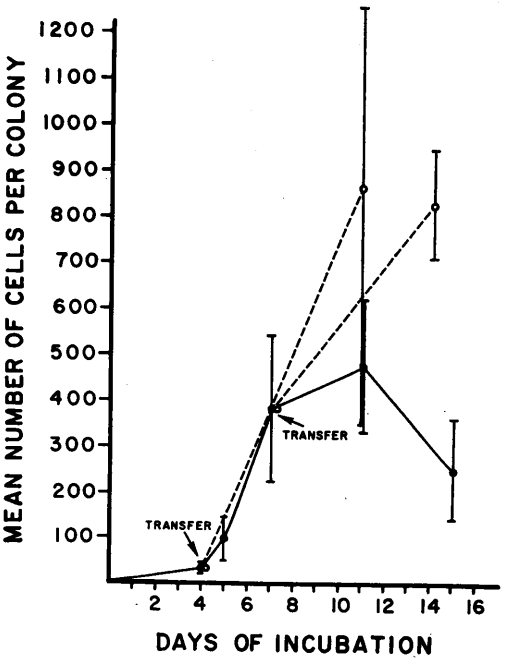

FIG. 1. Growth of DBA/1 bone marrow colonies stimulated by Swiss leukemic serum. Note superior growth of colonies after transfer to fresh plates. Vertical bars are standard deviations of data from 20 colonies at each time point.

larger size than control colonies left intact in the donor plates (Fig. 1).

Four-day colonies, initiated by leukemic Swiss serum, were transferred to plates containing 0.05 ml of leukemic Swiss sera of known colony stimulating activity, 0.05 ml of normal Swiss sera, pretested and found to lack colony stimulating activity, or plates with no added mouse serum. After 7 days further incubation on the transfer plates, colonies transferred to plates containing no added mouse serum or inactive normal Swiss serum usually either had failed to increase in size or showed a decrease in cell numbers (Table I). Colonies initiated by leukemic Swiss serum also were transferred to plates containing Moloney leukemic serum from BALB/c mice or serum from leukemic AKR mice which had been pretested and found to exhibit colony stimulating activity. Progressive growth of the colonies occurred on plates containing Moloney leukemic serum or leukemic AKR serum, but the size achieved by the colonies was smaller than that achieved by colonies on plates containing homologous

TABLE I. Growth of 4-Day Colonies Initiated by Leukemic Swiss Sera at 7 Days After Transfer to Plates Containing Various Types of Sera.

Type of serum in transfer plate	No. of different sera used	No. of colonies transferred	Mean No. of cells per colony*
No serum	—	24	32 ± 17
Normal Swiss	7	20	38 ± 11
Moloney leukemic BALB/c	4	16	377 ± 254
Leukemic Swiss	4	27	863 ± 530

* ± Standard deviation.
Mean size of 4-day colonies transferred = 29 ± 15 cells.
Mean size of 11-day colonies on donor plates = 480 ± 141 cells.

TABLE II. Growth of 4-Day Colonies Initiated by Leukemic Swiss Serum at 7 Days After Transfer to Plates Containing Various Types of Sera.

Type of serum in transfer plate	No. of different sera used	No. of colonies transferred	Mean No. of cells per colony*
No serum	—	22	12 ± 8
Normal Swiss	4	22	28 ± 19
Leukemic Swiss	4	28	159 ± 65
Leukemic AKR	4	17	180 ± 134
Normal human	2	14	4 ± 3
Human mononucleosis	5	31	306 ± 161

* ± Standard deviation.
Mean size of 4-day colonies transferred = 25 ± 8 cells.
Mean size of 11-day colonies on donor plates = 142 ± 50.
Mouse sera doses were 0.05 ml per plate and human sera doses were 0.2 ml per plate.

leukemic serum (Tables I and II).

Sera from some humans with leukemia or mononucleosis have been found to stimulate colony formation by mouse bone marrow cells. This work will be reported in full elsewhere. Colonies initiated by leukemic Swiss serum were transferred to plates containing 0.2 ml of inactive normal human sera or 0.2 ml of pretested mononucleosis serum known to exhibit strong colony stimulating activity. Colonies transferred to plates containing normal human serum failed to proliferate and most cells in the transferred colonies died during incubation (Table II). Colonies transferred to plates containing mononucleosis serum achieved a size greater even than colonies transferred to plates containing homologous leukemic Swiss serum.

In reciprocal experiments, (Table III)

TABLE III. Growth of 4-Day Colonies Initiated by Human Mononucleosis Serum at 7 Days After Transfer to Plates Containing Various Types of Sera.

Type of serum in transfer plate	No. of different sera used	No. of colonies transferred	Mean No. of cells per colony*
No serum	—	15	16 ± 15
Normal human	2	13	11 ± 8
Human mononucleosis	2	15	492 ± 320
Normal Swiss	2	10	38 ± 28
Leukemic Swiss	4	23	205 ± 116

* ± Standard deviation.
Mean size of 4-day colonies transferred = 30 ± 15 cells.
Mean size of 11-day colonies on donor plates = 385 ± 180 cells.
Mouse sera doses were 0.05 ml per plate and human sera doses were 0.2 ml per plate.

4-day colonies of DBA/1 bone marrow cells, initiated by human mononucleosis serum, grew progressively after transfer to plates containing human mononucleosis or leukemic Swiss serum but failed to increase in size on plates containing normal human or normal mouse serum.

All cells in transferred colonies were mononuclears, similar to those seen at 11 days in bone marrow colonies stimulated either by leukemic mouse serum or by active human mononucleosis serum. In none of the transferred colonies were granulocytic cells observed.

Growth of dispersed colony cells on transfer. The proliferative capacity of dispersed cells from 3-day colonies (containing mainly granulocytic cells) was compared with that of cells from 7-day colonies (containing mainly mononuclear cells). Since colony size is dependent on time of incubation and the dose of serum used(1,2) 3-day and 7-day colonies of comparable size (7-13 cells) were produced by reducing the serum dose used to stimulate the development of colonies sampled at 7 days. After 7 days of incubation the cell clusters, produced by the proliferative activity of the transferred cells, were removed and the cells counted and typed. With the methods used it was not possible to identify

TABLE IV. Growth on Transfer of Single Cells from Developing Bone Marrow Colonies.

Age of colonies at transfer	No. of cells in colonies	No. of single cells plated	No. of cell clusters after incubation	% "Cloning efficiency"	% of colonies failing to form clusters	Mean No. of cells in cell clusters
3 days	7–13	212	41	19%	40%	15 ± 13 (Range 3–64)
7 "	7–12	53	25	47%	0%	8 ± 4 (Range 2–17)
7 "	60	420	180	43%	0%	10 ± 8 (Range 2–40)

isolated single cells with certainty, but doublets and triplets were able to be counted and typed. With two exceptions, all cell clusters from both 3-day and 7-day colony cells contained only mononuclear cells. Forty-seven percent of 7-day colony cells but only 19% of 3-day colony cells proliferated to form clusters (Table IV). Clusters produced by 3-day cells were slightly larger than those of 7-day cells but cluster size was very variable and these differences were not significant statistically. Because of their small size, only a few of the cell clusters would have been scored as "colonies" in routine colony counts. Dispersal of larger 7-day colonies, containing 60 cells, indicated a similar efficiency in forming cell clusters as was noted for smaller 7-day colonies.

Cytological typing of typical 3-day and 7-day donor colonies revealed that only 20-30% of 3-day colonies contained mononuclear cells, whereas in 100% of 7-day colonies most cells were mononuclear although 20% also contained small numbers of granulocytic cells.

Discussion. The present results indicate that the colony stimulating factor in leukemic serum not only initiates colony formation but is required continuously for the progressive growth of these colonies. Cross reactivity exists between the colony stimulating factors present in the serum of leukemic Swiss, Moloney leukemic and AKR leukemic mice and, in addition, between the mouse leukemic serum factor and a similar factor present in human mononucleosis serum.

From the transfer studies using dispersed colony cells, it appears possible to clone individual colony cells. Cloning has also been achieved with colony cells derived from spleen cells(6). This technique should allow a further systematic analysis of the development of bone marrow colonies under *in vitro* culture conditions. Cells from older colonies (containing mononuclear cells) were more efficient in forming cloned cell clusters than were cells from early developing colonies (containing mainly granulocytic cells). This implies that under these conditions of colony stimulation, the granulocytic cells in developing colonies may not be capable of extended proliferation *in vitro,* a conclusion supported by the disappearance of these cell types from developing colonies after 6 days of incubation.

It is doubtful whether the small cell clusters, developing from dispersed colony cells can be regarded as true colonies of the type developing *de novo* from stimulated bone marrow cells. It is more likely that the small cell clusters represent merely the capacity of individual colony mononuclear cells to carry out a further limited series of divisions.

Summary. Transfer studies of developing *in vitro* mouse bone marrow colonies have shown that the colony stimulating factor in leukemic mouse serum is required both to trigger colony formation and for continued division of colony cells. Cross reactivity of colony stimulating activity was demonstrable between the sera of leukemic Swiss, Moloney and AKR mice and human mononucleosis serum. Transfer studies have demonstrated the feasibility of cloning developing bone marrow colony cells, the cloning efficiency varying from 19 to 47%. Cloning did not lead to the renewed formation of true colonies of the type developing *de novo* from stimulated bone marrow cells.

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Effect of Temperature Change on Heart Performance (Heart-Lung Preparation). (32564)

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In 1912 Knowlton and Starling(1) reported that within wide limits the output of the heart is independent of temperature in isolated heart-lung preparations. Since then many papers have been published on the cardiovascular effects of hypothermia, most of them on intact animals(2-6).

In an isolated heart-lung preparation, Reissmann and Kapoor(7) reported that lowering the temperature diminished the ratio of work to filling pressure. On the other hand, both Covino(8) and Goldberg(9) demonstrated a marked increase in myocardial contractile force, by attaching a strain gage arch to the myocardium of anesthetized, artificially ventilated, hypothermic, but otherwise "intact" dogs.

In this study of the influence of temperature on cardiac performance, an isolated heart-lung preparation was chosen in preference to an intact animal. This preparation excludes nervous and humoral influences; thus the changes observed may be ascribed to the direct action of temperature *per se* on myocardium. By reporting the effects on a stroke work basis, we are avoiding some of the complications of the chronotropic effects of temperature.

Method. Mongrel dogs (7.7-10.0 kg body weight) were anesthetized intravenously with sodium pentobarbital (30 mg/kg). The method of Knowlton and Starling(1) was followed with some modifications. The preparations were ventilated either with room air or with a mixture of 40% O_2, 4% CO_2, and 56% N_2. The preparations were supported by a continuous infusion of 5% glucose (10 mg/min) containing insulin (0.008 units/min) (10). Five mg heparin per 100 ml blood was used as an anticoagulant.

The blood reservoir was a polythylene bag 4 inches in diameter immersed in a water bath 9 inches in diameter to maintain a constant pressure head in spite of changes in volume of blood in the reservoir (Fig. 1). The inflow and the aortic pressure were adjusted with 2 screw clamps. The water bath was maintained thermostatically and altered rapidly by heating or adding ice. The blood temperature was read from a thermometer in the cannula in the superior vena cava through which the blood was delivered from the reservoir to the heart.

Arterial pressure was recorded through a polyethylene catheter inserted into the left subclavian artery and connected to a Statham P 23 Dd transducer and a Brush 8-channel oscillograph recorder. Left atrial pressure was recorded through a rubber tube inserted through the left appendage and connected both to a Statham PR 23 transducer and to a saline manometer; the latter was used for calibration and direct reading.

In some experiments right atrial pressure was measured by a saline manometer through a polyethylene tube inserted *via* the inferior vena cava.

The systemic flow was recorded with a rotameter(11). The pulmonary flow was measured with a pulsed field electromagnetic flowmeter (Medicon Division of Statham In-

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