doses of horse serum. It seems unlikely that the emboli observed in sensitized mice were air bubbles. Air emboli were observed in mice injected via the tail veins with saline containing air bubbles. Air emboli have thick, clearly visible refractile outer boundaries. In contrast, the outer edge of the embolus seen in anaphylactic animals was not at all distinct. In addition, air emboli were visible in the pulmonary circulation immediately after the injection of an air bubble into the tail vein. The emboli in anaphylactic mice did not appear until a few minutes after the challenge dose of antigen had been administered.

It should be mentioned that in the majority of cases administration of challenge doses of horse serum was not followed by death of the animal. Vasoconstriction, embolization and sludge were observed, but conditions returned to normal in a few minutes. In contrast, challenge doses of horse serum invariably killed 80 to 100% of pertussis-inoculated horse serum sensitized mice which were not subjected to the procedures of anesthesia, exposure of the lung and oxygen insufflation. It seems likely that the combination of anesthetic and oxygen had a protective effect in mouse anaphylaxis.

Discussion. Our observations of pulmonary vascular changes in mice during anaphylactic shock are very similar to the observations in rabbits and guinea pigs reported by Burrage and Irwin(1). Our results are also consistent with those of McMaster and Kruse(7) who described vascular changes in the ears of mice during anaphylaxis. The latter noted vascular spasm, clumping of cells and plasma skimming. It is possible that the pulmonary phenomena observed during mouse anaphylaxis account for the symptoms observed in the intact animal, namely cyanosis and respiratory embarrassment. At present the nature of the pulmonary emboli which have been seen is unknown. Our current hypothesis is that they are clumps of leucocytes sticking to an antigen-antibody precipitate.

Summary. Arteriolar constriction, clumping of red cells, and embolization have been noted in the pulmonary circulation of mice during anaphylactic shock.

We are indebted to Dr. Melvin H. Knisely for his encouragement and his many helpful suggestions.

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Received May 11, 1956. P.S.E.B.M., 1956, v92.

Sustained Propagation of Sarcoma 180 in Tissue Culture.* (22473)

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The delineation of the specific amino acid, salt and vitamin requirements of a mouse fibroblast (strain L) and of a human carcinoma (strain HeLa)(1-6) has permitted the continuous propagation of these cells in a medium containing only demonstrably essential growth factors, in which the omission of a single amino acid or vitamin, of glucose or serum protein, resulted in the cessation of growth and cell death. Eagle also has reported the successful isolation and propaga-

* This investigation was supported in part by research grants C-937 and C-2782, from National Cancer Institute of N.I.H., U.S. Public Health Service.

tion of a human epidermoid carcinoma (strain KB) directly from a biopsy specimen in these media(7). Several other normal and malignant human and animal cells also have been cultured directly from surgical material into media embodying these same minimal requirements supplemented with 10 to 20% whole serum, and a number of previously established cell lines have been similarly propagated(8-10). The purpose of the present report is to record the isolation and continuous propagation of Sarcoma 180 (S-180) in these ("HeLa") media directly from biopsy specimens.

Experimental. The medium, prepared as described by Eagle(1-7), contains 13 essential amino acids, 7 essential vitamins, glucose and salts, each in the concentration necessary for optimal growth of the HeLa cell. All experiments were done in duplicate series. The medium used in one series was supplemented with 10% human serum, while parallel experiments were done in media prepared with 10% horse serum. Similarly, the effect of various supplements to the basal substrate was studied in replicate experiments employing both media. Biopsy specimens of S-180 maintained in CFW mice† were minced with sterile scissors and suspended in 15-25 ml of complete growth medium. Trypsin,‡ 0.25% final concentration was added and the suspension incubated at 37°C on a "Magnimix" stirrer (adjusted to produce gentle agitation) to disperse the cells, as described by Youngner(11). The turbid supernatants containing dispersed cells were removed at 15-20 min intervals and stored in an ice bath. The bits of tissue remaining in the original flasks were resuspended in fresh media, and the trypsin-dispersing process repeated several times until no gross particles of tissue remained. The supernatants were pooled, centrifuged at 500-600 rpm for 3-5 min and washed 3 times with 10-15 ml of media to remove the trypsin. The washed cells were resuspended in media without serum and aliquots of the resulting heavy suspension were inoculated into 3 ml of complete medium in culture flasks(1,12) with a surface area of approximately 15 sq cm and incubated (stationary) at 37°C. For quantitative experiments, the washed cells were enumerated directly by haemocytometer counts, diluted appropriately and cultured in a similar manner.

TABLE I. Rate of Growth of S-180 in "HeLa" Medium.

Cell count × 10^4,* after hours incubation								
0†	24	48	72	96	120	144	168	192
6.0	6.6	14.7	33.0	60.2	105.4	116.0	151.3	203.5

Age of inoculum (days)	Cell counts × 10^4* Hr incubation 0	48	96
5	6	14.7	60.2
10	9	5.5	44.5
15	6	6.2	25.0

* Mean values of 2 experiments in replicate.
† Inoculum prepared from 5-day-old culture.

Results. The original cultures initiated in this manner on 16 June, 1955 grew poorly in media prepared with human serum, even when supplemented with 2% beef embryo§ extract. The cultures in media containing horse serum grew slowly at first, and there was a definite favorable response to beef embryo extract. The media were changed after 48 hrs and at 48 hr intervals thereafter, without disturbing the cells. After 11 days, the cell layer growing on the glass in the original cultures was exposed *in situ* for 1-2 min to 0.125% trypsin‡ (final concentration) in fresh complete media, the resulting free cells and cell clumps washed and subcultured in media containing 10% horse serum, with and without 2% beef embryo extract. The rate of growth continued to increase with successive transplants only in media containing beef embryo extract until after the 4th transplant on glass; thereafter beef embryo extract was no longer required. At this time, the cells were transplanted to 1-liter Blake bottles (surface area of approximately 150 sq cm) in media containing 10% horse serum and have been so maintained since that time with transplants

† This strain of Sarcoma 180 was obtained through the courtesy of Dr. F. Homburger, Cancer Research and Cancer Control Unit, Tufts University Medical School, Boston, Mass.

‡ Difco "1:250".

§ Microbiological Associates, Bethesda, Md.

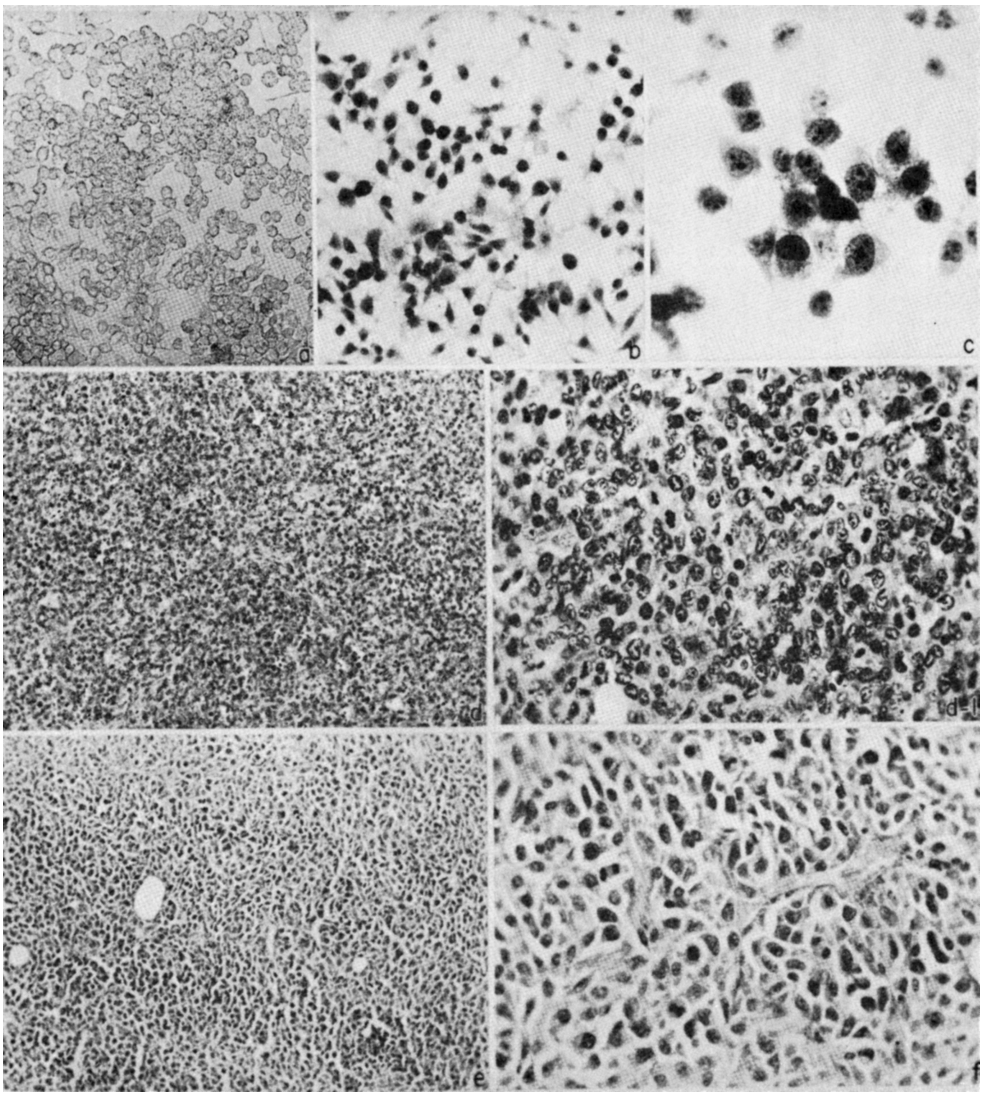

FIG. 1. (a) S-180, living, 6-day-old culture of 24th *in vitro* transplant growing *in situ* on glass. Unstained, ×137. (b) Same as (a). Collodian fixation, H & E, ×137. (c) Same as (b), ×417. (d) S-180 in CFW mice. 15-day tumor produced by usual trocar implant. H & E, ×137. (d-1) Same as (d), ×417. (e) S-180 in CFW mice. 15-day tumor produced by tissue culture inoculum of 2.5 × 10^5 cells in 0.1 ml media. H & E, ×137. (f) Same as (e), ×417.

at 5-7 day intervals.

The rate of growth of S-180 as determined in a typical experiment by haemocytometer counts is illustrated in Table I. The growth rate appears to have become stabilized at a generation time of *circa* 30 hrs in the logarithmic phase after 20 *in vitro* transplants, thus approximating that observed with the KB cell(7). Experiments on the effect of inoculum age on the lag and early logarithmic phase of the growth curve (Table I) indicate that the optimal time for transplantation is during the period of maximum metabolic activity. The microscopic appearance of the adherent, syncytial cell layer in living, glass-surface cultures is illustrated in Fig. 1a. The typical morphology of these cells in stained

TABLE II. Titration of S-180 Tissue Culture Inocula in CFW Mice.

Cells/inoculum*	Morbidity Tumors/Mice	%	Latent period, days Mean	Range
More than 10^5	20/20	100	3.5	3- 5
10^5	20/20	100	3.5	3- 5
10^4	16/20	80	8.6	6-13
10^3	7/20	35	19.7	16-27
10^2	2/20	10	29	29-34
10	2/20	10	29	29-34
10^{-1}	0/20	0		
Diluent control	0/20	0		
	ED_{50} data			
Expected	10/20	50	7	3-16
Observed (5×10^3 cells)	8/20	40	6.6	5-15

* Cells from 5-day-old cultures in volume of 0.1 ml subcut. in inguinal.

preparations is illustrated in Fig. 1b, c.‖

The cells obtained from trypsin-treated cultures of transplant numbers 5, 8, 17, 20, 23 and 26 after 38, 117, 165, 186, 200 and 215 days respectively *in vitro* produced typical sarcomas (Fig. 1d, e, f) following incubation periods of 3-5 days when implanted subcutaneously in CFW mice. Suspensions prepared from these tissue cultures can be quantitated and titrated in mice as has been done with tumor breis or ascites cells by several investigators(13-19), and the ED_{50} computed (20). The results of a typical experiment done with the 20th transplant (after approximately 60 generations *in vitro*) are summarized in Table II. The minimal inoculum resulting in tumors in 50% of the mice implanted has been observed repeatedly to be *circa* 4.5-5.0 x 10^3 cells, about 90% of which are viable as judged by the trypan blue technic(21). The time:dose relationship characteristic of decreasing inocula also can be used to predict within reasonable limits the incubation period following implantation, and the expected morbidity:time relationship agrees reasonably well with observed values. as illustrated by the typical experiment summarized in Table II.

Implantation of replicate inocula of 1.0 x 10^5 or more cells subcutaneously in CFW mice thus far has resulted in 100% morbidity, but the tumors so induced exhibit considerable variation in size (Table III). That this variation is due at least in part to differences in the growth rate of the tumor cells in different mice is indicated by the observation that inocula of 1.0 x 10^5 or more cells produce palpable tumors in all mice so injected within 3-5 days. The host factors determining these differences are as yet undefined, although there is some suggestion that tumors induced in female CFW mice are smaller than those in male mice (Table III). It is possible that variable immunological responses in individual mice tend to select from the inoculum cells of differing growth potentials. The isolation by *in vitro* technics of HeLa cells showing differing characteristics under various conditions of growth has been demonstrated recently(22). However the tissue culture sublines derived from small and large tumors by re-isolation *in vitro* thus far

TABLE III. Variation in Size of Tumors Induced by Replicate S-180 Tissue Culture Inocula in CFW Mice.

Inoculum*	Tumors/Mice	Days	Wt tumors in mg Range	Mean
23rd *in vitro* transplant				
10^5 cells	20/20	5	3- 32	10.5
	" †	10	8- 346	137
	"	15	15-1529	246
26th *in vitro* transplant‡				
2.5×10^5 cells§	(♂) 10/10	7	30- 159	87
	(♀) " †		38- 102	63
	Total		30- 159	75
	(♂) 10/10	14	102- 522	290
	(♀) "		51- 314	170
	Total		51- 522	230
5.0×10^5 cells	(♂) 10/10	7	60- 175	108
	(♀) " †		35- 106	72
	Total		35- 175	90
	(♂) 10/10	14	143- 683	375
	(♀) " †		123- 348	248
	Total		123- 683	311

* Cells from 5-day-old cultures in vol of 0.1 ml subcut. in inguinal.

† One tumor lost before weighing.

‡ Inocula prepared from untrypsinized cultures showed similar variations in tumor size.

§ Tumors induced by this inoculum suspended in 0.4% agar-agar exhibited similar variations in size.

‖ We wish to thank Dr. J. M. Craig, Department of Pathology, The Children's Medical Center, for these histological studies.

have exhibited similar variations in the size of the resulting tumors following implantation in CFW mice.

Discussion. The use of beef embryo extract (and other supplements) in the basal medium in the original experiments was prompted by earlier observations that S-180 and a variety of other animal and human tumors initiated growth but failed to survive serial subculture in the unsupplemented medium. The survival of S-180 and a spontaneous sarcoma of Syrian hamsters(8) in supplemented media suggests that for these cells the beef embryo extract provided the factor(s) required for survival which were either unavailable or deficient in the unsupplemented medium. Fischer(23) has suggested that cells implanted in artificial substrates may require additional nutritional factors until acclimated to their foreign environment. On the other hand, the epidermoid carcinoma isolated by Eagle(7), 5 Wilm's tumors, and a teratoma isolated from biopsy specimens(8) all grew vigorously when implanted in the unsupplemented medium. Whether or not the ultimate growth of S-180 in unsupplemented media represents a biochemical adaptation to the substrate cannot be stated at the present time.

S-180 is now well established in Blake bottle cultures and at this time is in the 32nd transplant, having undergone more than 100 generations during 230 days *in vitro* in these laboratories. This cell line also is being cultivated on a large scale for commercial distribution.§ The ready induction of specific amino acid and vitamin deficiencies in S-180 as has been described for other cell lines in these media(1-4), and their reversal by restoration of the missing metabolite, suggested the use of this cell line for the assay of the cytotoxic action of carcinolytic agents in tissue culture(24). Detailed experiments on the nutrition and cytology of S-180 in these tissue cultures are now in progress.

It is evident that these *in vitro* cultures of S-180 have thus far retained the capacity to induce typical sarcomas in CFW mice. Whether or not this characteristic will be maintained indefinitely is conjectural at this time; however two strains of human epidermoid carcinomas which have been maintained in these media for a much longer period of time induce typical epidermoid carcinomas when implanted into the cheek pouch of golden hamsters(25). The time:dose relationships observed with tissue culture inocula of S-180, like those reported with epidermoid carcinomas in hamsters(25), may prove to be useful in the quantitative study of potential chemotherapeutic agents. Tissue culture cells also present advantages as source inocula for the routine propagation of S-180 in mice, since such inocula are free of extraneous bacteria and are readily quantitated. Further experiments in this direction are now in progress.

Summary. 1. A strain of Sarcoma 180 maintained by routine trocar implant passage in CFW mice has been isolated and serially propagated *in vitro* from biopsy specimens in a medium containing only the growth factors demonstrably essential for the HeLa cell and the L strain of mouse fibroblasts. The generation time in these media is *circa* 30 hrs and this cell line is now in the 32nd transplant, having undergone more than 100 generations during 230 days *in vitro*. 2. Inocula prepared from these cultures after 215 days *in vitro* have produced characteristic sarcomata within 3-5 days in 100% of the CFW mice implanted subcutaneously with 1.0×10^5 or more cells. The ED_{50} in CFW mice is *circa* $4.5\text{-}5.0 \times 10^3$ cells, and the latent period between injection and the development of palpable tumors increases with decreasing inocula.

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Received May 11, 1956. P.S.E.B.M., 1956, v92.

Rate of Displacement of Oxytocic Substances from Dyencephalon to Tuber Cinereum in Hypophysectomized Rats. (22474)

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We reported(1) an increase in concentration of oxytocic substances in the tuber of hypophysectomized rats killed 5 to 14 days after hypophysectomy. The oxytocic activity, determined by its effect on uterus *in vitro*, was approximately 8 times higher in the tuber of hypophysectomized animals than in nonhypophysectomized controls.

In the present work we have attempted to determine the rate at which this change takes place by determining the oxytocic activity of tuber extracts obtained from hypophysectomized animals killed half an hour after hypophysectomy.

Method. Fifteen experimental sets of animals were used, each one of them included the following groups: 1) nonhypophysectomized animals; 2) animals killed half an hour after hypophysectomy; 3) animals hypophysectomized after death. Hypophysectomy was performed through the base of the skull in animals anesthetized with avertin by intraperitoneal injection, the anesthesia lasting 20 minutes. All animals were anesthetized and killed in a gas chamber (death in about 3 minutes) when the effects of anesthesia had disappeared. The sets included animals of the same sex, weight, and age, each group containing 4 animals, with a total of 59 nonhypophysectomized and 67 in each one of the other groups. Separate extracts were prepared immediately after the animals were killed using equivalent amounts of tissue from the tuber. Tissue from 4 animals belonging to the same group was ground with micronized quartz sand and 0.5 ml of water (acidified to pH 3) per 200 g of rat weight was added. The extract was centrifuged at 2000 rpm for 15 minutes. The oxytocic activity was determined on a uterus of a rat in diestrus immersed in Tyrode solution prepared according to Gaddum with the addition of 2 mg of atropine per liter(2). Previously neutralized extracts were added to the bath in volumes ranging from 0.1 to 0.5 ml. The oxytocic activity of the unknowns was obtained by proper dilution and comparison with a standard solution of commercial oxytocin ("Pituitrol") containing 10 u. per milliliter.

Results. A higher oxytocic activity of the tuber region was found in animals hypophysectomized and then killed than in animals hypophysectomized after death. Only in 4