TABLE II. TGT Substrate Clotting Times (Sec.) 1 and 7 Min. after Incubation of Untreated, Heated and Frozen Control and 7 Min. Clot-Lysed Platelets.

	Untreated		100°C, 10 min.		Frozen-thawed	
	TGT incubation time (min.)					
Platelets	1	7	1	7	1	7
Control	40+	9.8	40+	10.0	40+	9.8
7 min. clot-lysed	23.0	13.0	40+	10.0	21.0	10.8

TGT substrate clotting time, perhaps due to destruction of contaminating thrombin.

It was also noted that the platelets from the early phases of clotting did not contain full plasma-thromboplastin activity as both $BaSO_4$ treated plasma and serum were necessary for their full activity in the TGT test.

Comment. These experiments show that "platelets", even after clotting, retain their full platelet-thromboplastic or factor 3 activity. After clotting and clot lysis the platelets have a shaggy appearance but are still recognizable with the phase microscope. It is possible that the thromboplastic activity is present in small granules outside of the platelet shell which sediment at the same speed, although such granules could not be distinguished microscopically. The chopped ground clot sediment had no ability to retract clots. The sediment from the lysed clotted samples, even after many washings, caused lysis of plasma clots. Additional experiments showed that human platelets contain a streptokinase cofactor (? proactivator) and when added with streptokinase to bovine profibrinolysin produce an active fibrinolysin.

Platelets obtained from recalcified platelet-rich plasma in the early stages of clotting retain traces of thrombin. They are able to clot platelet-poor plasma without added calcium and, in the presence of calcium, produce very rapid clotting. The same platelets when used in a TGT test promote lessened evolution of plasma thromboplastin unless they are first diluted or heated. This apparent inhibition is reminiscent of the poor evolution of plasma thromboplastin observed by Spaet(4) when very concentrated platelets were used. The anticoagulant activity of Spaet's platelet lipid was stable at 100°C., while that found in defatted platelets was destroyed by heat. The apparent anticoagulant activity of platelets separated from lysed samples soon after recalcification is destroyed by 100°C, leaving only the coagulant activity.

Summary. Sediments obtained from platelet-rich plasma allowed to clot for 1 hour contain full platelet-thromboplastic or factor 3 activity. Sediments obtained from recalcified platelet-rich plasma in the early phase of clotting contain thrombin and appear to be inhibitory to normal thromboplastin generation.

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The Cytostat, An Apparatus for Automatically Controlled Continuous Propagation of Suspended Cells.* (26947)

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Designs for continuous cultivation of cells in suspension have been introduced particularly for the study of bacteria, algae and

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fungi. Myers and Clark(1), Bryson(2) and Anderson(3) described the *turbidostat* principle for continuous cultivation of bacteria. Here, as turbidity increases in proportion to cell density, fresh medium is supplied while an equivalent quantity of cell suspension is removed.

A different principle of cell cultivation is used in the *chemostat,* introduced by Monod (4) and at about the same time by Novick and Szilard(5). Here medium is supplied continuously to the cell culture and an equivalent quantity of cell suspension is removed simultaneously. Rate of flow of the medium is so adjusted as to make the culture self-limiting and to achieve a *steady-state.* A prerequisite for this control is that the medium contains a limit concentration of some essential metabolite.

Attempts have also been made to propagate suspended mammalian cells of several types *in vitro.* Owens, Gey and Gey(6) described how they cultivated lymphoblastic mouse tumors in rotating tubes. Earle *et al.* (7) reported success in growing L-fibroblasts in suspension in flasks mounted on a Brunswick agitator. Cherry and Hull(8.9) and McLimans *et al.*(10) described the stirrer or spinner culture, where cells are agitated by a rotating magnetic stirring bar suspended by means of a nichrome wire. Interesting results were obtained with a large number of established mammalian cell lines. In these cases, however, the entire culture medium or a large part thereof was changed at various intervals (batch principle). A steady-state of growth was never achieved or attempted.

It is uncertain whether the turbidostat has been successfully adopted for controlled growth of mammalian cells. The chemostat, however, has been used for propagating Earle's L-fibroblasts and a simplified chemostat for growth of mammalian cells was recently introduced by Cohen and Eagle(11), who described the characteristics of continuous growth of the cell line HeLa-S3, KB and liver. A similar principle was utilized in the so called cytogenerator of Graff and McCarty (12).

In the rapidly developing field of cell culture *in vitro* two trends are clearly seen: (1) interest in meeting future undefined needs to grow mass cultures of mammalian and other cells; (2) the necessity for obtaining basic information about cell reactions to environment(13) particularly under steady-state conditions.

The turbidostat is useful for a number of applications in bacteriology. However, in a medium with distinct optical properties, determination of concentration of cells with light scattering surface will have to depend on complex relationships between scattered, transmitted and absorbed light. For regulation or direct readings of cell concentration the turbidostat principle is therefore not ideal.

The chemostat has the advantage of simplicity but operates under conditions of some growth-limiting metabolite which may be comparable to cell starvation. It is not known to what extent the limiting factor changes the metabolism of the cell and how the cell, grown under such conditions, compares with a cell propagated in an environment with no other limitations than those inherent with the cell.

To study this and other related problems the *cytostat* (I.R.L.2) was developed. It permits use under conditions when either the cell concentration or a limiting essential metabolite concentration is used as the controlling factor. Intermittent readings of cell density can be made and growth comparisons of a number of simultaneous cultures are possible. It has also proved itself useful for pilot experiments for purposes of mass propagation in steady-state. These functions are carried out automatically. Fig. 1 shows the cytostat in which the turbidometric principle was replaced by a nephelometric device. As this construction has proved a useful aid to a number of *in vitro* studies of cell populations, a description is given.

The Cytostat comprises a heat-insulated Plexiglass housing which can accommodate up to 8 silicone-treated culture vessels containing 50-1500 ml. Nutrient medium is fed by tube to each culture vessel and the volume of each individual culture is regulated by an adjustable drainage tube which maintains constant level by withdrawing cell suspension

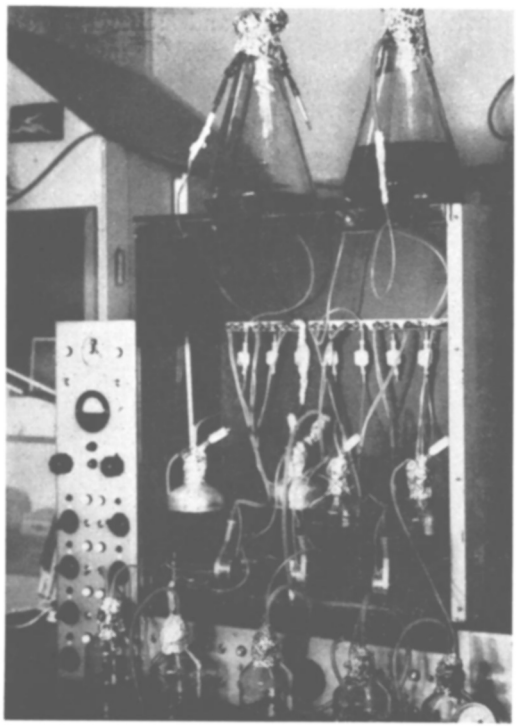

FIG. 1

as soon as the supply of fresh medium raises the level above the drainage tube. Rotating magnets provide cell suspension agitation, a double thermostat and simmerstat maintain constant temperature, a fan circulates air in the Plexiglass housing and individual gas circulators pump suitable gas through the culture vessels.

When used as a chemostat, the medium is pumped continuously into the culture vessels. When, however, cell concentration is used as the controlling factor, *a nephelometric unit,* (Fig. 2) is used. This consists of *a circular light source and a photocell,* (Fig. 3) and is placed on *a cylindrical projection on the wall of the culture vessel* (Fig. 4) and a sensitrol relay is set to operate at a given cell concentration. A rheostat controls the intensity of the light falling on the cells and the photocell-rheostat system can be used to read cell concentration at chosen intervals after suitable calibration.

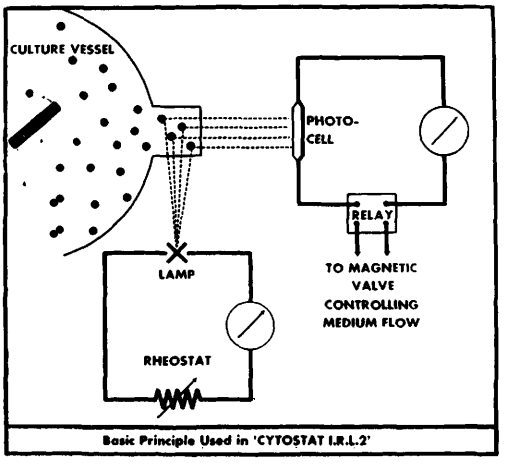

FIG. 2

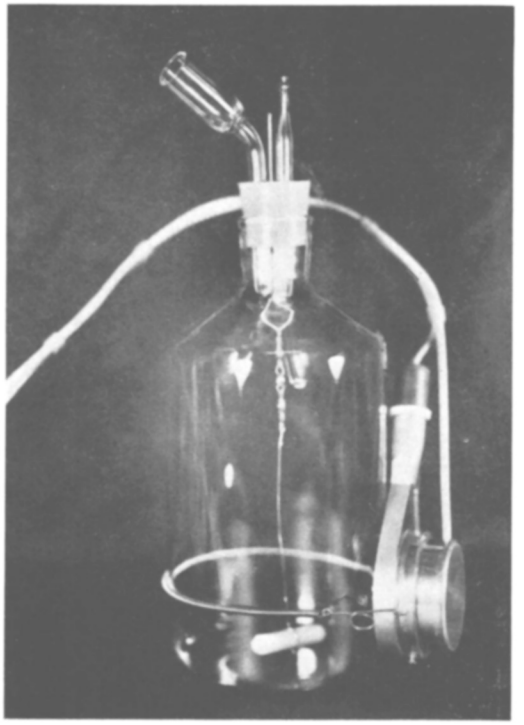

FIG. 3

A programmed, automatic reader (Fig. 5 and 6) records hourly the cell concentration of each culture vessel. Whenever cell density exceeds a set limit, a silicone-treated magnetic valve opens to allow addition of fresh medium to the culture until it is diluted to the set concentration. An equivalent quantity of cell suspension is simultaneously removed by the partial vacuum created in the drainage tubes by the gas circulators.

The critical detail is the photocell light-source sensitrol-relay combination. Dowex

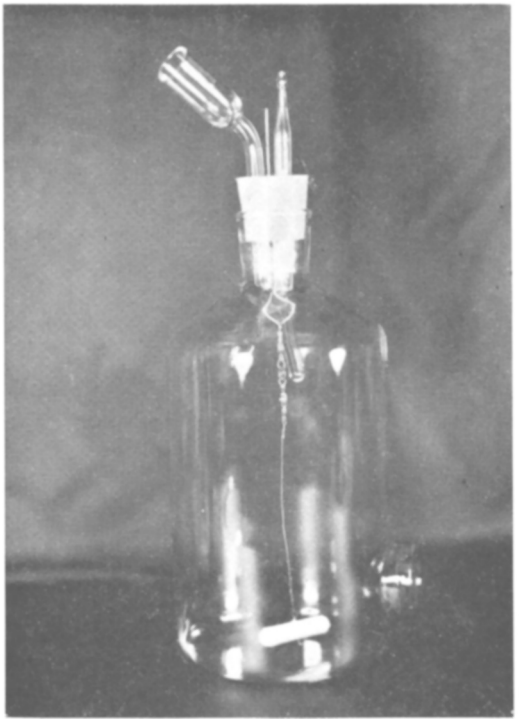

FIG. 4

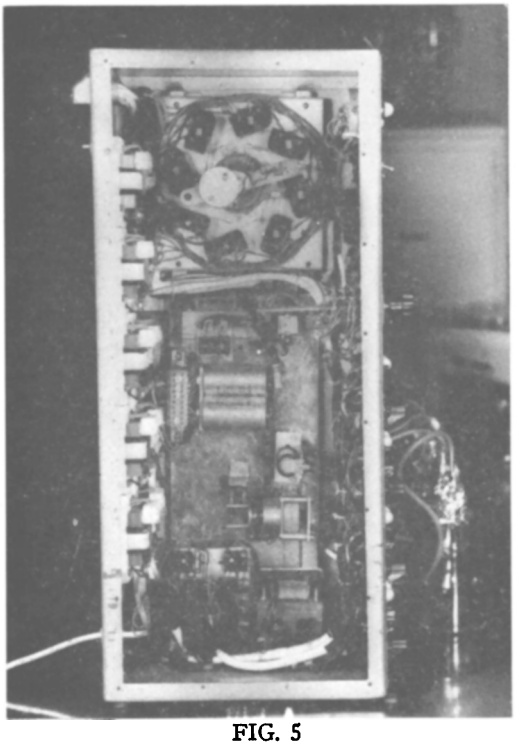

FIG. 5

particles, red blood cells and HeLa cells were used to test it. Fig. 7 shows the intensity of the incident light (lamp voltage) as a function of the cell density of the suspension at

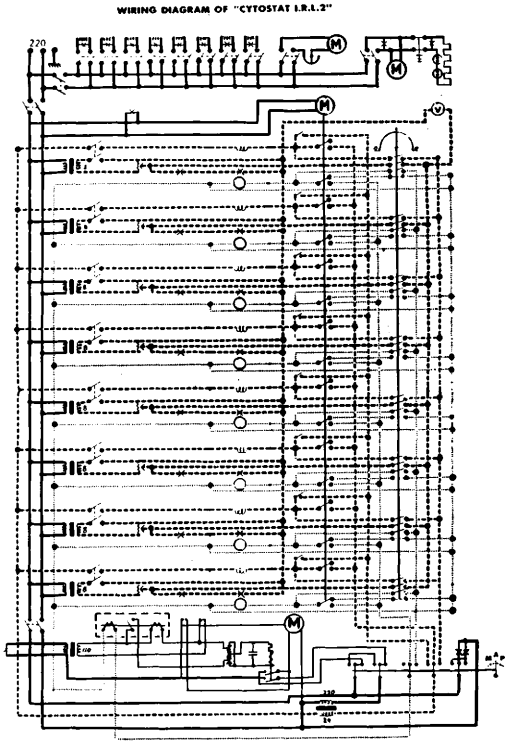

FIG. 6

a constant scattered light. Readings were made at 2 different intensity levels. Even though Dowex particles reflect light comparatively weakly, it is apparent that

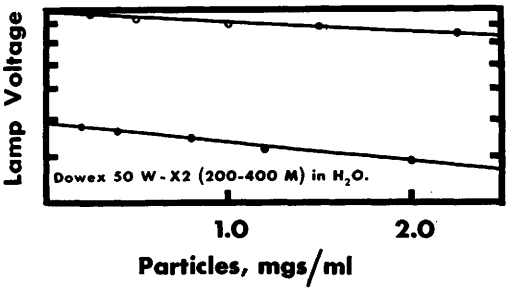

FIG. 7. Two levels of incident light versus cell concentration at constant scattered light.

straight-line graphs are obtained at both levels and that they are almost parallel if the logarithmic scale is taken into consideration. It is also apparent that light intensity, meas-

ured here as a function of lamp voltage, plays no deciding role.

Fig. 8 shows how much more strongly human red blood cells scatter incident light than do Dowex particles. The intensity level of the scattered light may be kept constant either by using high cell concentration or high lamp voltage.

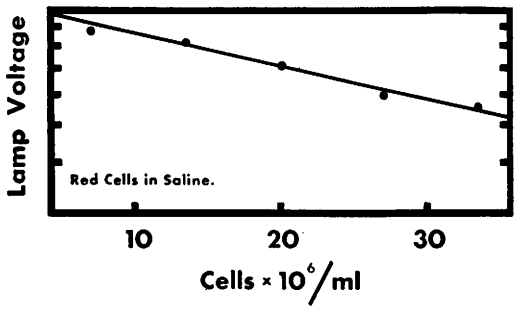

FIG. 8. Incident light versus cell concentration at constant scattered light.

The relation between incident light and cell concentration at a constant level of scattered light is not influenced to any special degree by a serum content of up to 40% of the medium or addition of phenol red to the medium (Fig. 9). But because different photocells react differently, it is important that the type chosen be especially sensitive to light in the yellow and red spectrum and not particularly dependent on ultra-violet light, which is absorbed by proteins.

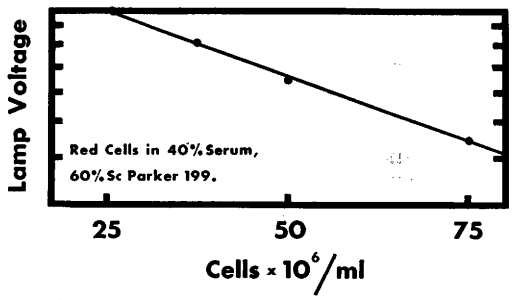

FIG. 9. Incident light versus cell concentration at constant scattered light.

A comparison of HeLa cell and human red blood cell suspensions shows that either type of cell can be used for nephelometric cell concentration determinations, whether in balanced saline or in serum-containing media. Fig. 10 shows the practically parallel straight-

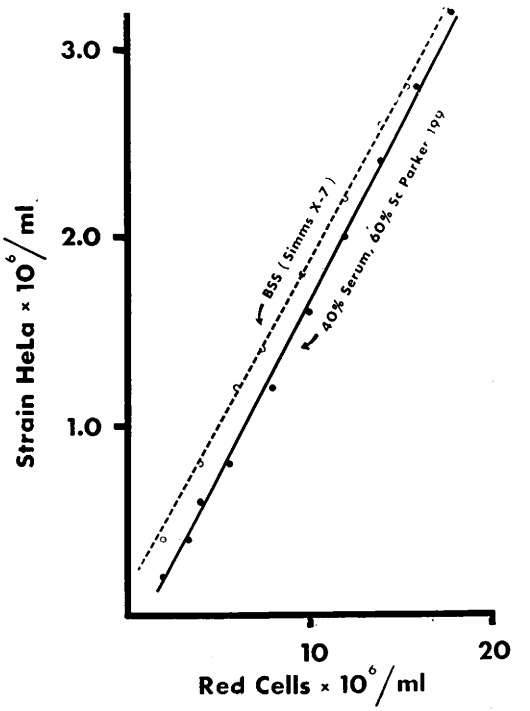

FIG. 10. Iso-nephelometric relations of human red cells and HeLa.

line relationship between HeLa cells and red blood cells, measured nephelometrically, and

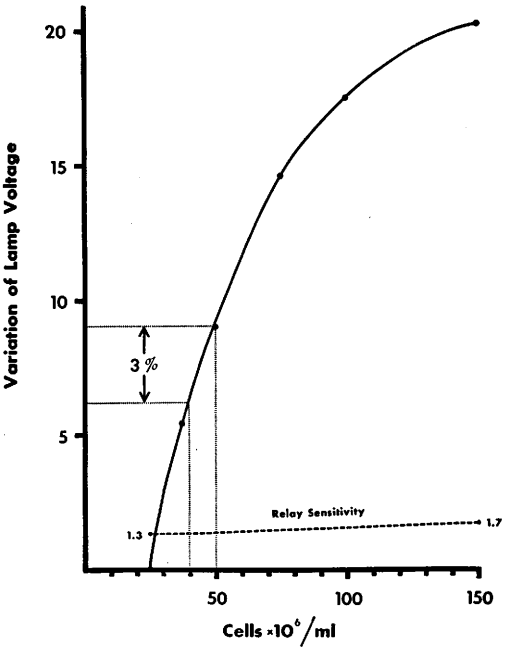

FIG. 11. Percentage variation of incident light versus cell concentration at constant photocell output.

that a HeLa cell scatters as much light as 5 or 6 red blood cells. The latter can therefore be used for checking and adjusting the optical system.

The sensitivity of the control system may be judged from Fig. 11 which shows that a 3% alteration of the incident light is necessary to maintain a constant level of scattered light if the cell density increases from 40 to 50 million/ml. Since the sensitivity of the sensitrol relay is better than 1.5%, it is clear that such an increase of cell density cannot occur without the relay operating and supplying fresh medium. Within this concentration range, the system has a sensitivity to change of about ± 5%.

Summary. The cytostat and its operation have been described. It would seem to be a useful aid to further *in vitro* studies of cell populations.

The authors owe thanks to Mr. Tage Axelsson and Mr. Artur Andersson for skillful and devoted contributions.

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Recovery of Reoviruses from Wild and Laboratory Mice. (26948)

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Representatives of the ECHO 10 family, recently designated as the reovirus group(1), have been isolated from man, chimpanzees, monkeys, and cattle, and serologic evidence of infection has been detected in guinea pigs and other mammalian species(1,2). This report describes the isolation of naturally occurring viruses serologically related to reovirus types 2 and 3 from wild mice and laboratory mice, respectively.

Materials and methods. Tissue cultures. Primary trypsinized mouse embryo (ME) and rhesus monkey kidney (MK) tissue cultures were obtained from Microbiological Associates, Inc., Bethesda, Md. Maintenance fluid for ME cultures was 5% horse serum in Eagle's Basal Medium, which was replaced twice weekly. MK cultures were maintained in Mixture 199, without intercurrent fluid change; in later tests, 1% calf serum without gamma globulin (Newborn Agamma calf serum, Hyland Labs., Los Angeles, Calif.) was added. All media contained penicillin (250 u/ml) and streptomycin (250 μg/ml).

Mice. For experimental inoculations, weanling and newborn Swiss mice, strains "General Purpose" and "NIH", were obtained from the Animal Production Section, Nat. Inst. of Health. Mice tested for virus were sacrificed within 24 hours after receipt in the laboratory. Wild mice were live-trapped.

Viruses. As reference viruses, the following reovirus strains were used: type 1, SV