TABLE II. Hypoglycemic Convulsions Produced with Insulin Eluted from Powdered Glass.

10 ml insulin sol. in contact with glass			10 ml acidified saline wash		10 ml 5% U.S.P. gelatin			Control insulin in 5% gelatin		
						Gelatin from glass				
Conc., μg/ml	ml recovered	μg left on glass	ml recovered	μg left on glass	Max not draining, μg/ml	ml	Mice in convulsions	μg/ml	ml	Mice in convulsions
30	9.4	18	10.2	1	0.1	1.0	3/3	4	1.0	0/3
						.3	1/3			
35	9.0	35	10.2	2.5	0.25	.25	15/17	4.5	.25	10/10
						.10	2/10	4.5	.10	3/10
								.5	.25	0/17

did not cause convulsions. Evidence that the convulsions were hypoglycemic was obtained by administering 10% glucose intraperitoneally to mice in convulsions and terminating the convulsions. Three mice bled during convulsions had blood sugar values below 45 mg %. To obtain some idea of quantity of insulin eluted from the glass by the gelatin, a solution of 4.5 μg/ml insulin diluted with 5% U.S.P. gelatin in acidified saline was administered in the same dose ratio as the gelatin from glass in the second experiment of Table II. The results are similar to those obtained when gelatin from the glass was used. This suggests that the gelatin from the glass contained several μg/ml. The difference in insulin sensitivity of the mice used in the 2 experiments of Table II is probably owing to size and strain difference.

Conclusions. Our results indicate that material having hypoglycemic activity is adsorbed to glass from a solution of insulin and may be eluted from it with a solution of 5% U.S.P. gelatin. Since adsorption of proteins to glass is a well known phenomenon and since the hypoglycemic activity of insulin has never been dissociated from the intact molecule, it is safe to assume that insulin is adsorbed to glass and may be eluted from it. Under proper conditions a 5% solution of gelatin will inhibit adsorption of insulin to glass.

Summary. By elution with gelatin solutions a material having hypoglycemic activity in mice can be recovered from laboratory glassware which has previously been exposed to insulin. Gelatin solutions can prevent adsorption of insulin to glass.

1. Ferrebee, J. W., Johnson, B. B., Mithoefer, J. C., Gardella, J. W., *Endocrinol.*, 1951, v48, 277.
2. Newerly, K., Berson, S. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 751.
3. Hill, J. B., *Endocrinol.*, 1959, v65, 515.
4. Lostroh, A. J., Jordan, C. W., Jr., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 267.
5. Somogyi, M., *J. Biol. Chem.*, 1945, v160, 69.
6. Park, J. T., Johnson, M. J., *ibid.*, 1949, v181, 149.
7. Saifer, A., Gerstenfeld, S., *J. Lab. and Clin. Med.*, 1958, v51, 448.

Received June 19, 1959. P.S.E.B.M., 1959, v102.

Choriocarcinoma of Women Maintained in Serial Passage in Hamster and Rat. (25149)

ROY HERTZ
Natl. Cancer Inst., Bethesda, Md.

The heterologous transplantation of malignant tumors of man was initially reported by Greene(1). Toolan(2) and Sommers *et al.* (3) described methods for increasing the frequency of success of such heterologous tumor grafts through prior suppression of the immune response of the animal host by x-irradiation and by cortisone administration. Pierce *et al.* described the biological behavior of human testicular tumors carried in the hamster

cheek-pouch(4,5). We employed similar procedures in the adaptation of 3 choriocarcinomata of women to serial transplantation in the hamster. One of these tumors has now also been maintained in serial transfer in the rat. This report will describe the biological and hormonal features of these tumor strains. We named our tumors the Erwin-Turner Choriocarcinoma.* Strains WO, BO, and MA.

Materials and methods. Tumor tissues used were derived from 3 women with a histopathologically established diagnosis of choriocarcinoma according to morphological criteria of Novak and Seah(6). The original tissues for strains WO and MA were obtained at autopsy from cerebral metastases. Strain BO was derived from a nodular metastasis which had been surgically resected from patient's right breast. All 3 patients had shown an initial therapeutic response to methotrexate(7,8). However, patients who provided tissue for strains WO and MA had previously become methotrexate-resistant. The patient who provided the tissue for strain BO continued to show a limited degree of response to methotrexate. Female golden hamsters of NIH strain varying from 1-3 months in age were employed. Intact and hypophysectomized female Sprague-Dawley rats weighing 45-55 g were also used. All animals were maintained at room temperature between 76° and 80°F. Purina checkers were fed as basal diet. Hypophysectomized rats received ground-meat supplement daily and hamsters received daily ration of kale, carrots, and apples. Starting with original human tissue, transfers have been made by inoculation into the hamster cheek-pouch by means of freshly excised piece of tissue about .05 cu cm in volume. Rat transfers have all been made subcutaneously in the right flank area. Aseptic precautions have been applied throughout. Nembutal anesthesia was employed. Recipient hamsters were given 3 mg of cortisone acetate in aqueous suspension at time of inoculation and every third day subsequently during ensuing 2 weeks. Recipient rats received the same dosage schedule of cortisone for a 3 week period and were also exposed to general body irradiation just prior to inoculation, as recommended by Toolan(2). Repeated appraisal of qualitative and quantitative development of tumor inocula were made by direct inspection and by free-hand sketch of size and shape of enlarging tumor mass. Histological studies were performed on selected specimens by fixation in Bouin's solution, paraffin sectioning and staining with hematoxylin and eosin. At autopsy, careful evaluation of quantitative and qualitative hormonal effects of tumor

TABLE I. Transfer Generations of WO Strain of Erwin-Turner Choriocarcinoma in Hamster and Rat.

Transfer generation	Date of transfer	No. of animals used	No. of takes after 7 days	Recipients
1	10/24/58	20	15	Cortisonized hamsters
2	11/10	35	22	
3	26	35	19	
4	12/18	50	24	
5	23	20	17	
6	1/ 8/59	80	52	
7	1/13/59	33	18	Hamsters (no prior treatment)
8	22	25	20	
9	30	40	32	
10	2/ 9	20	13	
11	16	25	20	
12	24	25	14	
13	3/ 4	25	16	
14	11	20	17	
15	19	30	25	
16	26	72	49	
17	4/ 2	21	18	
18	9	24	16	
19	16	46	39	
20	23	59	42	
21	30	24	17	
22	5/ 7	42	36	
1	10/24/58	10	3	Rats (cortisonized and irrad.) hypophysectomized (H) or intact
2	11/10	20	4	
3	21	20	6	
4	12/10	30	10	
5	20	20 (H)	10	
6	1/ 6/59	20 "	12	
7	21	10 "	6	
8	28	12 "	7	
9	2/ 6	22 "	10	
10	20	11 "	4	
11	3/ 3	20 "	12	
12	13	20 "	11	
13	25	22 "	9	
14	1/ 3	17 "	7	
15	14	22 "	10	
16	24	22 "	10	

* These tumors are named in grateful acknowledgement of efforts of Mr. Howard L. Erwin and Mr. Charles K. Turner without whose diligence and technical skill this valuable material would not have become available.

transplant upon various endocrine organs were recorded.

Results. There are listed in Table I the actual transfer generations of 1 of the tumor strains (WO). Time intervals may be calculated from dates of respective transfers. The almost immediate adaptation of this strain to the heterologous host may be noted. Transfers were usually made when the tumor had reached an estimated volume of 1-1.5 cc.

Growth of each tumor strain has assumed a characteristic course. The WO strain has proven to be the most readily adaptable strain in both hamster and rat. It quickly exhibited a high percentage of takes and a very rapid course. Transfers are now readily made at weekly intervals and the tumor runs its full course to spontaneous necrosis and liquefaction in 15-20 days. Moreover, it has now been carried in serial transfer for 12 generations in the hamster without use of cortisone. This makes the strain especially useful in studies of endocrinological relationships. In the rat, however, the WO strain still failed to grow unless the host had been irradiated and had also been treated with cortisone. In addition, tumor growth in the rat was considerably slower and spontaneous necrosis occurred between 10-15 days following inoculation.

In the WO strain the initial phase of host response is noted in hamster cheek-pouch within 2-3 days. This consists of formation of a turbid exudate about the tissue inoculum. In the following 3 days this is rapidly replaced by highly vascularized, bluish-purple tissue which then extends and rapidly fills the cheek-pouch. Maximum size of 1-1.5 cc is usually reached by about 10 days and during ensuing 5-10 days the tumor loses its bluish color and becomes successively pink, grey, and finally greenish yellow. At this point, the necrotizing mass may slough and drain and by 30 days following inoculation, only a small residual scar may be found in the cheek-pouch. Less frequently the liquefied tumor mass is indefinitely retained. In this strain 60-80% takes are regularly noted at one week following inoculation and an additional 10-15% of inoculated animals will develop a growing tumor during second week.

The remaining 2 strains, BO and MA, continue to require cortisone for survival. They also have a smaller percentage of takes and their growth progress is about ⅓ as rapid as that observed in the WO strain. Nevertheless, their qualitative behavior is quite comparable in all respects to that already described for the WO strain. It seems that heterologous adaptation of these 2 strains is not yet as complete as is that of the WO strain. The BO strain has been transferred 28 times in 22 months and the MA strain has been transferred 12 times in 7 months.

Histological study of all 3 strains reveals a close identity of the morphology of the heterologous transplant with that of the original patient material. The rich vascularization of tissue is its most noteworthy feature. The marked tendency to hemorrhagic necrosis of extensive areas of the tumor is consistently observed. Cytotrophoblast and syncitial trophoblast are formed into broad sheets and abundant mitoses are observed throughout. Marked cellular pleomorphism is characterized by very frequently occurring bizarre, multinucleated cellular and acellular masses. On the whole, the picture represents one of rapid, disorganized growth with extensive hemorrhage and necrosis.

Nevertheless, in no instance have we observed microscopic evidence of actual cellular infiltration into the wall of the hamster cheek-pouch. Moreover, no instance of distant metastasis has been observed. It is especially noteworthy that this highly proliferative and densely vascularized tumor tissue readily peels away from the surrounding host tissues with practically no hemorrhage at points of separation.

The peripheral endocrinological effects of the 3 tumor strains in the hamster are quite comparable (Table II). The ovaries of recipient animals show extreme gonadotropic stimulation characterized by 3-5 fold enlargement consisting of marked follicular and luteal stimulation. Their uteri, Fallopian tubes, and vaginae are extremely hyperemic and grossly enlarged. That this effect is secondary to the ovarian stimulation is shown by total absence of such effect in the previously ovariectomized recipient. Hence it may be inferred that these tumor strains are not directly productive of

TABLE II. Endocrine Effects of Human Choriocarcinoma in Female Hamsters and Rats.*

Species	Status	Tumor	No. of animals	Body wt	Ovaries	Uterus	Adrenals	Thyroid
				(mg)				
Hamster	Intact	+	25	73 ± 8	143 ± 30	641 ± 80	12 ± 2	
		—	10	82 ± 5	27 ± 3	276 ± 76	10 ± 1	
	Ovariecto-mized	+	30	102 ± 6		32 ± 6	10 ± 2	
		—	10	100 ± 7		30 ± 4	10 ± 1	
Rat	Intact	+	15	65 ± 9	230 ± 40	312 ± 80	23 ± 6	5 ± 2
		—	6	50 ± 4	10 ± 2	18 ± 2	9 ± 3	3 ± 2
	Hypophysec-tomized	+	18	49 ± 5	44 ± 6	83 ± 12	10 ± 2	3 ± 1
		—	10	50 ± 4	6 ± 1	17 ± 3	9 ± 2	3 ± 1

* All values are for Erwin-Turner choriocarcinoma, Strain WO. All rats were previously cortisonized and irradiated. All hamsters previously untreated; data are from one representative series.

estrogenic steroids in the heterologous host (Table II). The tumor-bearing hamster also exhibits no gross or microscopic evidence of thyrotropic, lactogenic or adrenotropic effect.

Similarly, the WO tumor strain exhibits only gonadotropic action in either the hypophysectomized or intact rat. Lactogenic, adrenotropic, or somatotropic potentialities cannot be readily evaluated in the rat because of complicating peripheral action of high doses of cortisone given these tumor recipients. Thyrotropic effect is entirely lacking. The marked quantitative and qualitative difference in gonadotropic effect in the hypophysectomized rat versus the intact rat indicates that the gonadotropic action of this tumor is entirely comparable with that considered to be characteristic for human chorionic gonadotropic hormone(9) (Table II).

Gonadotropic potency can be demonstrated in homogenates of heterologously maintained tumor tissue as well as in the peripheral blood of the tumor-bearing hamster by the use of infantile mice as test animals. As little as 2 mg of fresh tumor tissue or 1 cc of peripheral blood will induce maximum uterine enlargement and ovarian stimulation in an infantile mouse. Further studies will be required for a more detailed quantitative estimate of the actual hormonal output of these several tumor strains.

The growth pattern of each respective tumor strain has proven to be highly reproducible from generation to generation. Our method of recording of these growth processes by the use of free-hand, life-size, sketches of the tumors at various time intervals is admittedly crude. Nevertheless, these growth-records are sufficiently quantitative to permit gross evaluation of the effect of various inhibitory agents upon the course of tumor growth.

Summary. 1) Three choriocarcinomata from women have been successfully adapted to serial transplantation in cheek-pouch of the cortisonized hamster and one of these can be carried in previously untreated hamster. This latter strain has also been adapted to subcutaneous growth in the cortisonized, irradiated, hypophysectomized or intact female rat. 2) These heterologously maintained tumors produce in the host gonadotropic effects characteristic of human chorionic gonadotropic hormone. Biologically detectable amounts of hormone are readily demonstrable in homogenates of the growing tumor tissue and in peripheral blood of tumor-bearing hamster. The tumors exhibit no estrogenic, adrenotropic, or thyrotropic effect in the hamster. 3) Each tumor strain presents a quantitatively reproducible growth pattern which renders it adaptable to studies of the effect of chemotherapeutic and other inhibitory agents.

1. Greene, H. S. N., *Cancer,* 1952, v5, 24.

2. Teolan, H. W., *Cancer Research,* 1953, v13, 389.

3. Sommers, S. C., Chute, R. N., Warren, S., *ibid.,* 1952, v12, 909.

4. Pierce, B., Verney, E. L., Dixon, F. J., *ibid.,* 1957, v17, 134.

5. Verney, E. L., Pierce, G. B., Dixon, F. J., *ibid.,* 1959 (in press).

6. Novak, E., Seah, C. S., *Am. J. Obst. and Gynec.,* 1954, v67, 933.

7. Li, M. C., Hertz, R., Bergenstal, D. M., *New*

Eng. J. Med., 1958, v259, 66.

8. Hertz, R., Bergenstal, D. M., Lipsett, M. B., Price, E. B., Hilbish, T. F., *J.A.M.A.*, 1958, v168, 845.

9. Loraine, J. A., *The Clinical Application of Hormone Assay*, Livingstone, London, 1958.

Received June 22, 1959. P.S.E.B.M., 1959, v102.

Sensitivity of Populations of Clonal Lines of HeLa Cells to Polioviruses.* (25150)

GRACE LEIDY, KATHERINE SPRUNT, WINIFRED REDMAN AND HATTIE E. ALEXANDER
Babies Hospital (Presbyterian Hospital) and Dept. of Pediatrics, Columbia University, College of Physicians and Surgeons, N. Y.

Fluctuations in growth potential and susceptibility to invasion by a given virus in HeLa cell "farm" populations have been a common experience. In addition to these changes, we have also experienced emergence of cells which had a rapid growth potential and which failed to take up neutral red adequately for the detection of plaques. Our own experience with these periodic changes suggests that they occur without any known alterations in environment which might select certain types; the degree of change at times necessitated interruption of our studies. In an attempt to obtain HeLa cell lines which exhibit greater stability of growth potential and virus susceptibility, populations were propagated from single clones isolated by the method of Puck (1). Six presumably clonal lines and a number of subclonal lines were isolated from a "farm" population of HeLa cells and examined for their sensitivity to types I and II polioviruses. The cell lines were found to differ in their sensitivity; two of the most sensitive have been maintained for two years and the above fluctuations have not recurred to an appreciable degree. Variation in susceptibility to poliovirus among clonal strains of HeLa cells has been reported and the variation appears to be a stable character(2).

Materials and methods. "Farm" population. The "farm" HeLa cell population, originally obtained from Dr. Scherer, has been carried with weekly passage in a variety of media. The medium used for the present study is that modified by Puck, *et al.*(3). It was sterilized by Seitz filtration. *Isolation of clonal lines.* The technic of isolation of single clones was that described by Puck, *et al.* 24-hour old subcultures of the cells were trypsinized for 10 or more minutes (until populations were comprised mostly of single cells) and, after appropriate dilution in the growth medium, were plated in 60 x 15 mm Petri dishes. The plate preparations, placed in containers that could be made air-tight, were gassed for a few minutes with 5 per cent CO_2 in air and then incubated at 37°C for 9 to 11 days. In estimating the percent of single cells in trypsinized suspensions, clumps of no more than 2 cells were considered to be incompletely divided cells and were grouped as single cells. On this basis, 90 per cent or more of the populations plated were single cells. Some of the trypsinized parental clonal populations used for the isolation of subclonal lines were virtually 100 per cent true single cells. The plating efficiency of all but one of the parental clonal lines at the time of subclonal isolation was between 30 and 65 per cent. Plating efficiency *per se* was not explored for the different clonal populations. Colonies of cells were selected for isolation on the basis of gross microscopic morphology and adequate separation from adjacent colonies. Selected clones were ringed by cylinders as described by Puck *et al.*, trypsinized, and then transferred to 16 x 150 mm test tubes containing 1 ml of growth medium. After adequate growth on the glass, the cells were transferred to 2-oz bottles in 3 ml of medium and subsequently to 5-oz bottles in 10 ml of medium. In general, single clone populations were first tested for

* This work was supported by grants from Natl. Inst. of Health.