ketosis(6).

The insignificant rise in blood glutathione concentrations coincident with sharply falling blood glucose levels in the diabetic patients indicates that this convenient potential index of intracellular sulfhydryl activity is unaffected by the arylsulfonylurea compounds. Nevertheless, the essentially unchanged glutathione values do not entirely erase the possibility that metabolic activity of these agents may be mediated by altering the intracellular availability of sulfhydryl groups. Experimental work(11) demonstrates that profound changes in tissue concentrations of glutathione may or may not be accompanied by variations in the blood glutathione level. A similar situation may also exist in man.

Summary. 1. Blood glucose and reduced glutathione determinations were performed at intervals on 9 non-diabetic patients and on 15 patients with moderately uncontrolled diabetes following oral administration of 3 g of tolbutamide or carbutamide. 2. Mean maximal decrease from fasting blood glucose value was 14% in patients with normal carbohydrate metabolism, and 43% in the diabetic group. 3. In control patients there was no change in glutathione levels during the test period. In diabetic patients a minimal, insignificant rise had occurred 5 hours after ingestion of the hypoglycemic agent. 4. The mean blood reduced glutathione concentration was strikingly lower in patients with diabetes than in those whose carbohydrate metabolism was intact. In 4 patients subnormal values persisted despite restoration of normal fasting blood glucose levels by daily therapeutic doses of tolbutamide.

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Human Chorion Cells: Cultivation and Susceptibility to Viruses.* (23126)

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The ready availability and usefulness of amnion cells in tissue culture virology have been demonstrated since the original publication of Zitcer *et al.*(1). Modifications of the amnion cell culture, virus spectrum, and application to primary isolation have been reported by several groups(2,3). The chorion is a placental by-product of every amnion trypsinization, and therefore the usefulness of the chorion was explored.

Materials and methods. Chorion cell cultures. The methods employed were essentially similar to procedures used for the amnion(2). Fresh membranes were collected within one

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hour after delivery into Mason jars containing non-refrigerated Hanks' balanced salt solution (BSS) with antibiotics in concentrations described below. The chorion was separated from the amnion with forceps, and washed 4-6 times in Hanks' BSS. This procedure revealed a relatively blood-free membrane 2-3 times the thickness of the amnion, smooth and pink on one side and rough, yellow and opaque on the other. The tissue was then cut into approximately one cm squares for digestion in 0.25% trypsin and Hanks' BSS while kept in suspension by a magnetic stirrer. After one hour, supernatant fluid was discarded; the chorion was minced finely, and re-exposed to fresh trypsin solution at room temperature for 12-20 hours. The resulting supernatant which contained dispersed chorion cells was filtered through gauze and centrifuged for 10 minutes at 1200 rpm in a horizontal International Size 2 centrifuge. The cell pack was then washed twice in tissue culture medium with approximately 5% calf serum. One membrane generally yielded 1.0-1.5 ml cell pack, which was dispersed 1:125 in Eagle's basal medium(4) with 10% horse serum heated at 56°C for 30 minutes. Five-tenths ml amounts were dispensed into each stationary culture tube and incubated at 37°C. After cell attachment at 48 hours, the nutrient medium was replenished. A cell sheet generally formed in 4-6 days and the horse serum was reduced to 4%. Cultures were changed with 1 ml fluid once a week and could be maintained for 30 days. All fluids used contained 100 units penicillin, 100 μg achromycin, 50 units mycostatin, and 100 μg streptomycin/ml. *Chorion susceptibility to viruses.* Various viruses, usually prototypes of standard strains in use at the NIH, were employed throughout. One tenth ml of each virus pool was inoculated into 2 chorion tubes. These cultures were observed daily for appearance of maximal cytopathic effects. Supernatant fluids were then harvested and stored at −50°C until further passage. Cultures showing no cytopathic effects were followed for 14 days on primary chorion passage, and during later passages for 7-10 days. In subsequent passages 0.1 ml of the supernatant fluid from the previous chorion culture

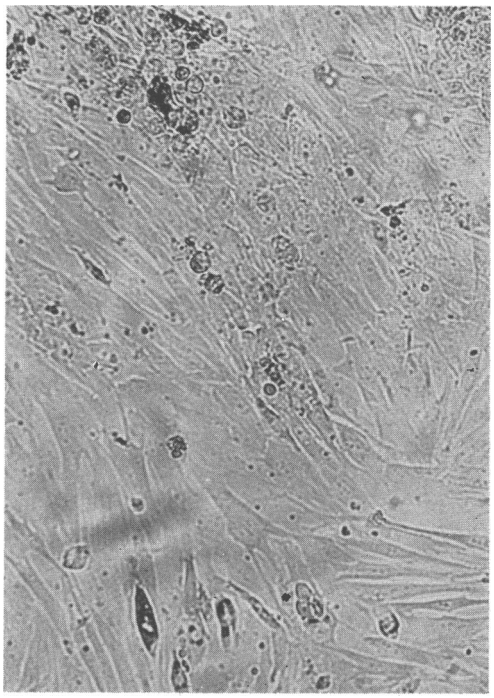

FIG. 1. Normal chorion culture (unstained) 5 days old showing an area composed largely of fibroblast-like cells (magnification × 150).

was transferred. All viruses, except salivary gland virus(5), tested were carried through a minimum of 5 tissue culture passages. Each 5th chorion passage was further inoculated into an appropriate susceptible host system, and those viruses producing cytopathic effects in chorion cells were found to produce pathologic changes in these different host systems compatible with each respective virus. Cultures inoculated with human salivary gland virus were incubated for as long as 17 days before first cytopathic effects were noted. When this change was marked, the adhering cells were scraped from glass with a rubber policeman, and passage was made with cells suspended in supernatant fluid. Salivary gland virus obtained from Dr. W. P. Rowe (5) was carried through 2 consecutive chorion passages, reisolated in human embryonic skin-muscle culture, and passaged once again in chorion. Pools of representative strains of those viruses that induced cytopathic changes in chorion were prepared by inoculating several chorion tube cultures with 0.1 ml fluid from the 5th, or in one case the 6th, chorion

TABLE I. Chorion Cell Susceptibility to Viruses.

Virus	Type	Virus source	Cytopathic effects: Epithelial cells	Cytopathic effects: Fibroblast cells
Poliovirus	1,2,3	Monkey kidney	+	+
Coxsackie B	1,2,3,4,5	*Idem*	+	±*
Coxsackie	A9	"	+	+
Adenovirus(7)	1,2,3,4,5,6,7,9†	HeLa or KB	+	±
Herpes simplex	—	Mouse brain	+	+
ECHO(6)	1,3,4,6,11	Monkey kidney	+	±
Salivary gland virus (5)	—	Human embryonic skin muscle	—	+
Influenza	A, B	Monkey kidney	—	—
"	C	Egg	—	—
Mumps	—	"	—	—
ECHO	2,7,8,9,10,12,13,14	Monkey kidney	—	—
Rabies	—	Mouse brain	—	—

* ± indicates cytopathic effect in fibroblast cells was not consistent on passage.
† On repeated trials adenovirus type 4 (RI-67) has not produced cytopathic changes beyond the second transfer.

passage. Titrations using these prepared 6th or 7th chorion passage pools as inocula were then performed with 4 tubes per 10-fold dilution. Chorion and at least one other susceptible tissue culture system were used.

Results. In the freshly prepared chorion cell suspensions 2 types of rounded cells were seen, a larger one about the size of a human polymorphonuclear leucocyte and a smaller cell approximately the size of a red blood corpuscle. After incubation of 4-6 days a sheet of mixed epithelial-like and fibroblast-like cells was formed (Fig. 1).

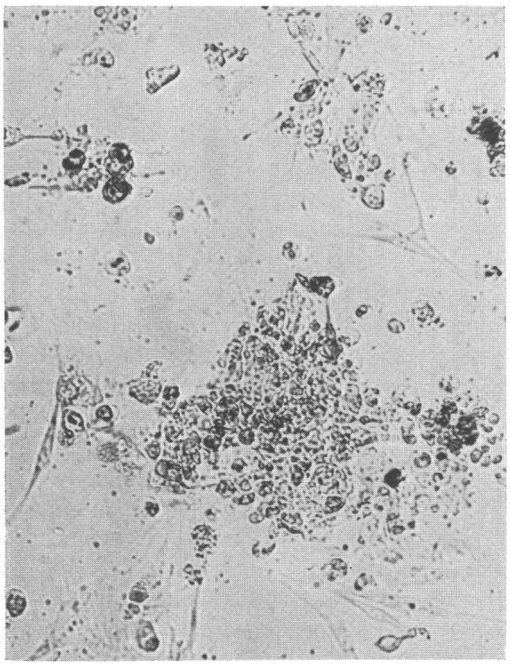

FIG. 2. Chorion cell culture (unstained) 60 hr after inoculation with poliovirus type 1 (magnification × 150).

The virus spectrum for the viruses tested is shown in Table I. Polioviruses types 1, 2, and 3 produced firstly cellular hypergranularity and rounding, and secondly, cell destruction in both epithelial and fibroblast cells (Fig. 2). The cytopathic effect of Coxsackie A9 could not be distinguished from that of the polioviruses. All 5 Coxsackie B and ECHO(6) viruses 1, 3, 4, 6, and 11 produced rounding of epithelial cells sparing many of the fibroblasts (Fig. 3). The adenoviruses (7) induced characteristic cellular clumping, and nuclear granularity seen also in HeLa and monkey kidney cell cultures, but the chorion fibroblasts were irregularly involved in adenovirus infection. Cell clumping and cytoplasmic stranding without marked nuclear hypergranularity were usually seen within 24 hrs. after inoculation with herpes simplex (Fig. 4). Salivary gland virus affected only the fibroblast cells of the culture which became enlarged, rounded, and opaque; first, only in an initial focus, but after 4-6 days, the entire cell sheet showed this effect. Influenza viruses A, B, and C, mumps virus, ECHO viruses 2, 7, 8, 9, 10, 12, 13, 14, and rabies virus produced no cytopathic changes, and subsequent inoculation into a known susceptible system revealed no virus.

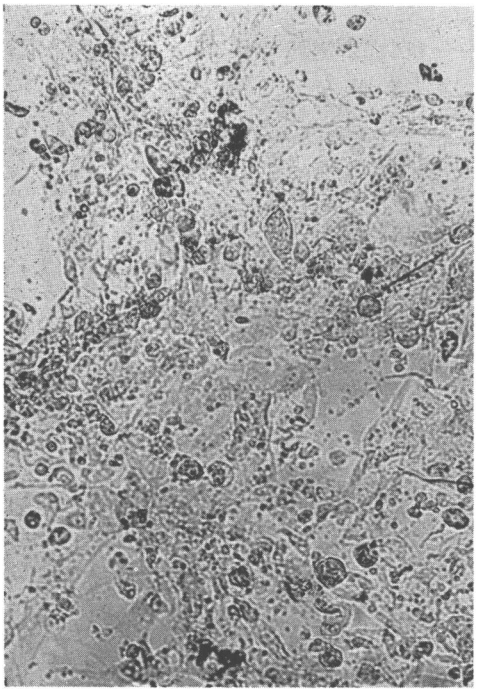

FIG. 3. Chorion cell culture (unstained) 5 days after inoculation with ECHO-6 virus (magnification × 150).

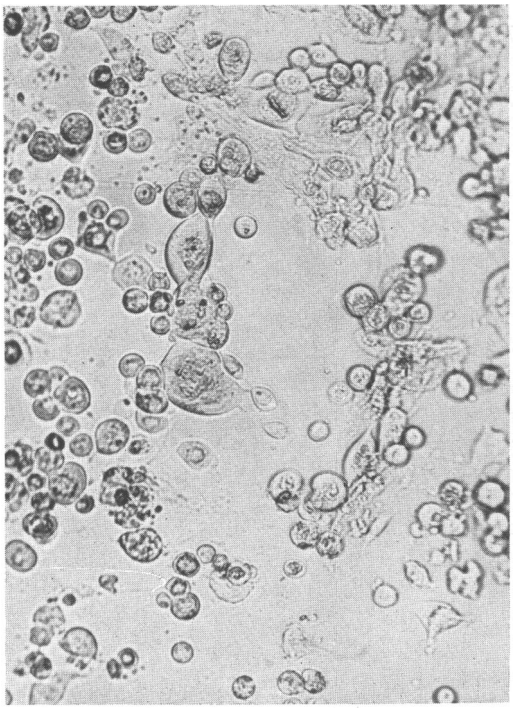

FIG. 4. Chorion cell culture (unstained) 72 hr after inoculation with herpes simplex virus (magnification × 150).

Limited work with vaccinia indicated that cytopathic effects in both epithelial and fibroblast cells are also regularly produced with this virus.

Table II shows the tissue culture infectious dose-50 ($TCID_{50}$) end-points with representative viral strains of known titer, namely poliovirus-1(10^6), Coxsackie B-3(10^5), Coxsackie A9($10^{5.5}$), herpes ($10^{2.6}$), adenovirus-3($10^{3.5}$), and ECHO-6(10^6) viruses. The results indicated that there had been propagation of these viruses in chorion cell cultures, and titers obtained in chorion were comparable to titers obtained in the other tissue culture systems tested with the same inoculum.

Discussion. Chorion cell cultures have been shown to be a ready and feasible source of mixed epithelial and fibroblast elements. The virus spectrum of epithelial cells was similar to that of the HeLa and human amnion cells. A single amnion usually yields approximately 250 tissue culture tubes, but by separate processing of the chorion from the same placenta, the number of available cultures can be increased by another 250. Moreover, the fibro-

TABLE II. Representative Titrations of Some of the Viruses Inducing Cytopathic Changes in Human Chorion Cells.*

Virus	Consecutive passages in chorion	Chorion	Amnion	Monkey kidney	HeLa
Poliovirus-1	6	4.3		4.8	
Coxsackie B3	6	4.5		6.0	4.3
A1	6	3.7		3.7	
Herpes	7	3.7	3.7		
Adenovirus-3	6	1.7		1.5	
ECHO-6	6	2.0		3.5	

* $TCID_{50}$/0.1 ml calculated by Reed-Muench method after observation period of 10 days.

blasts of the chorion offer the possibility of recognizing viruses which do not grow in epithelial cells, such as salivary gland virus. The fibroblasts have been maintained with infrequent medium changes for more than a month. The fact that chorion cultures contained 2 types of cell made recognition of cytopathogenic effect more difficult than with cultures of a single-cell type. After 2-3 weeks the epithelial cells were often shed leaving a relatively pure culture of fibroblasts.

Summary. 1. Chorion cell cultures of mixed epithelial and fibroblast-like cell types have been grown regularly in tissue culture. 2. Susceptibility of these cells to various viruses has been explored. Polioviruses types 1, 2, & 3; Coxsackie B1, B2, B3, B4, and B5; Coxsackie A9; adenoviruses 1, 2, 3, 4, 5, 6, 7, and 9; herpes simplex; ECHO viruses 1, 3, 4, 6, and 11; vaccinia; and human salivary gland virus have been found to induce cytopathic changes in chorion cell cultures.

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Some Normal Laboratory Values in the Dog. (23127)

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As part of preclinical evaluation of a potential drug in our laboratories the effect of long-term administration of the agent is observed in dogs. Because it is essential to know whether changes in chemistry or hematology are developing in response to a challenge with the therapeutic agent, control data are obtained on dogs prior to initiation of the agent. A compilation of these control data is presented herein. There is in the literature little systematic information concerning clinical chemical or hematologic norms in the dog in spite of extensive use of this species in laboratory work. Albritton(1) has made a critical selection of blood values from the literature, but many of the data were based on small populations, and each component was usually measured in a different group of dogs maintained under different conditions of diet and handling. This paper presents data obtained on blood and urine components in a large number of "normal" dogs under certain specified conditions. It is to be hoped that this may permit more useful correlations than would a mere compilation of the results of various tests that are at present scattered throughout the literature.

Methods. Female mongrel dogs were selected from stock on the basis of apparent good health, were dewormed, inoculated against distemper and maintained in metabolism cages for several weeks. A 500-g portion of food (25% horse meat in commercial dog food) was made available for one hour each day, after which remaining food was removed and weighed. Water was available at all times. After the dogs had become accustomed to handling and were of constant or increasing weight, weekly chemical and hematologic values were obtained prior to the daily meal. An extra 500 ml of water was given by gavage on the day preceding chemical analysis to insure an adequate volume of urine. Blood was drawn from the jugular