Table 1. Effect of ouabain on contractile response of rat myocardium to glucose and pyruvate.

Substrate	Ouabain concn. (mM)		Percentage of initial amplitude*				
			Minutes after addition of substrate				
			0	5	10	30	60
Glucose	0	(12)†	49 ± 2	36 ± 5	40 ± 5	51 ± 8	59 ± 8
Glucose	0.034	(7)	50 ± 4	57 ± 9‡	60 ± 9‡	74 ± 6‡	81 ± 6‡
Pyruvate	0	(8)	50 ± 3	61 ± 9	76 ± 14	105 ± 10	111 ± 9
Pyruvate	0.034	(6)	51 ± 4	68 ± 8	79 ± 10	97 ± 10	97 ± 14
Substrate-free control	0	(7)	51 ± 1	50 ± 2	48 ± 3	41 ± 3	33 ± 3
Ouabain control	0.034	(6)	50 ± 1	44 ± 4	40 ± 5	30 ± 5	19 ± 4

* Mean values ± standard deviation. † No. of experiments in parentheses. ‡ Significantly different from response to glucose in absence of ouabain [$p < 0.01$ (t test)].

tricle of the rat heart were stimulated in substrate-free phosphate medium at 27°C until the force of contraction had declined to approximately 50 percent of the initial amplitude (*3*). Ouabain was added at this point and, after the positive inotropic action had subsided to the 50-percent level, the substrate under investigation was added. The response was compared with that of strips to which substrate was added at the 50-percent level in the absence of cardiac glycoside. Ouabain increased the force of contraction to approximately 75 percent of the initial amplitude; the force declined again to the 50-percent level in approximately 15 minutes. This transient increase in the force of contraction produced by ouabain appeared to be related to depletion of endogenous substrates and was in marked contrast to the prolonged positive inotropic effect that occurred when the cardiac glycoside was added to slightly hypodynamic preparations (91 percent of the initial amplitude) or to ventricle strips that had become hypodynamic after prolonged contraction in a medium containing glucose. (The positive inotropic response at 37°C has been reported in a previous communication, *4*.)

The responses of the ventricle strip to 5.5 mM glucose in the presence and absence of ouabain are shown in Table 1. A noteworthy difference is seen in the immediate response, with a marked depression of force within 5 minutes in the absence of ouabain in contrast to the steady increase occurring in the presence of the drug. Although the force of contraction subsequently began to increase slowly in the absence of ouabain, the value attained at the end of the experimental period was still considerably less than that attained in the presence of the drug. On the other hand, a steady increase in force which was not influenced significantly by previous exposure to ouabain occurred following the addition of 2 mM pyruvate. Results similar to those with pyruvate were obtained with 2 mM β-hydroxybutyrate (16 experiments).

The fact that glucose was much more effective in sustaining the contractile activity of myocardium in the presence of ouabain than in its absence is in accord with Wollenberger's findings (*1*). It seems clear that the conversion of glucose to pyruvate is affected in view of the failure of the drug to alter the contractile response to pyruvate or β-hydroxybutyrate, or the rate of oxidation of pyruvate (*1*). This finding provides support for the view that the positive inotropic action may be based, at least in part, on an increased energy production by the heart.

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Isolation and Propagation of Rabbit Kidney Epitheliallike Cells

Simplification and refinement of tissue-culture procedures during the last few years have greatly aided viral research studies. Scherer, Syverton, and Gey (*1*) have discussed the need for established cell strains which will support the cultivation of viruses. It is the purpose of this report (*2*) to describe the successful isolation and propagation of a new cell type derived from rabbit kidney tissue (strain RbK). The strain is of potential interest because of its possible susceptibility to poliomyelitis and other viral agents.

On 28 Nov. 1956, both kidneys were removed from an adult, female, New Zealand white rabbit. The capsules were removed and minced kidney cortex (1 to 2 mm fragments) was prepared in Dulbecco's phosphate-buffered saline (*3*). The fragments were washed repeatedly with buffered saline until a clear supernatant was obtained. A cell suspension was prepared from the minced tissue according to the simplified method described by Bodian (*4*). Minced tissue was treated with 0.25 percent trypsin in Hank's balanced salt solution for a total of 24 hours at 5°C and washed twice with balanced salt solution, and the cells were resuspended in 5 ml of growth medium. The latter consisted of Eagle's basal medium prepared in Hank's balanced salt solution, 20 percent rabbit serum and 20,000 units of pencillin, 5 mg of dihydrostreptomycin, and 5000 units of mycostatin per 100 ml. One-tenth-milliliter amounts of undiluted cell suspension and of a 1/10 dilution were added to 0.9 ml of growth medium in Leighton tubes; tube cultures were set up in triplicate. In addition, 1 ml of undiluted cell suspension was added to 4 ml of medium in each of two culture flasks with a surface area of approximately 24 cm^2. All cultures were incubated at 37°C. Seventy-five percent of the culture fluid was renewed every third day.

Microscopic examination of the cultures showed that many cells had adhered to the glass during the first 72 hours of incubation. However, it was observed that growth progressed at a slow rate and was not stimulated by renewal of nutrient fluid. A predominance of spindle-shaped cells was seen in all cultures, and the cells were not in close apposition.

Ten days after the initial planting, the cells in the flask cultures were resuspended at 37°C in 5 ml of Hank's balance salt solution containing 0.25 percent trypsin, centrifuged at 600 rev/min for 10 minutes, and the supernatant was discarded. After resuspension in 5 ml of growth medium, the cells were transferred to clean, sterile flasks and returned to the incubator. No enhancement of growth was observed following this treatment, and the individual cells continued to appear spindle-shaped. On the eighth day the medium was discarded from the culture flasks and replaced with growth medium containing 5 percent bovine embryo extract. The cultures received a 75-percent renewal of nutrient fluid with embryo extract every third day.

Within a week a marked growth response and alteration in cytology were noted. The cells became polyhedral, and populations of contiguous cells were es-

tablished in the flasks. Hematoxylin- and eosin-stained preparations of cells grown on coverslips in culture tubes showed round or oval basophilic nuclei with numerous mitotic figures and large amounts of cytoplasm (Fig. 1).

The dramatic change in the appearance of the cells after 5 days in medium containing bovine embryo extract (23rd day of cultivation) suggested to us that the extract definitely influenced cell transformation. A culture of the original spindle-shaped cells which was not exposed to embryo extract did not show a change in cell type and was, eventually, discarded on the 41st day of cultivation. In a recent publication by Westwood, Macpherson, and Titmuss (*5*) the authors discuss various phases of cell type change. They note that in their cultures of embryo rabbit kidney tissue, fibroblasts were always present when transformation occurred. Transformation occurred spontaneously between the 26th and the 65th day of cultivation and could not be induced by any specific factors in the preliminary treatment of the tissues or in the treatment of the cells at subculture.

Our strain of rabbit kidney cells was serially subcultured in Eagle's basal medium containing 5 percent embryo extract and 20 percent rabbit serum for a total of 118 days. At this time, two out of four cultures were transferred to the same medium but without the addition of embryo extract. It was found that the epithelial cell type remained unchanged after repeated passage in the absence of extract; therefore, the use of embryo extract was discontinued. At the present time, the cells are being cultivated in Eagle's basal medium, as is described at the beginning of this report. The RbK cells have undergone 37 successful passages in this laboratory and have been grown in quantity without difficulty. Stationary cultures grow as well as rotated cultures.

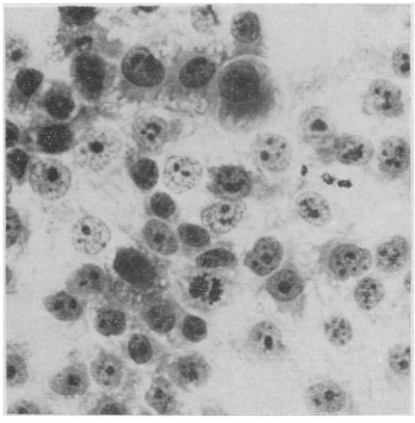

Fig. 1. Stationary culture of rabbit kidney epitheliallike cells from the fourth subculture in medium containing bovine embryo extract. Hematoxylin-eosin (× 160).

The susceptibility of the RbK strain to several viruses is being investigated. Preliminary results with poliovirus are encouraging. This agent has been grown serially in RbK cells through several transfers with titers comparable to those obtained in monkey kidney. Furthermore, it causes a cytopathology which manifests itself by an early clumping of the cells (1 to 3 days), followed by their rapid and complete sloughing off the glass (3 to 5 days) (*6*).

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6. The viral tests are being carried out by Victor Cabasso of the Viral and Rickettsial Research Division of the Lederle Laboratories, Pearl River, N.Y. It is a pleasure to acknowledge this valuable contribution to the work.

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Isotope Effects in Gas-Liquid Chromatography

In view of the widespread use of gas-liquid partition chromatography, it seems timely to draw attention to the considerable changes in retention volume which may result from extensive substitution of deuterium or tritium for hydrogen in organic compounds. Such isotope effects, while they increase the difficulty of identifying labeled compounds, provide a measure of the relative vapor pressures of the isotopic compounds and may, therefore, be used to estimate the number of tritium atoms per molecule of a substance that is present in trace quantities.

Figure 1 shows the separation of cyclohexane and cyclohexane-d_{12} (*1*) peaks obtained at 53°C with a 4-meter didecyl phthalate column and a flow rate of 45 ml (STP) of helium per minute, using a Perkin-Elmer vapor fractometer (model 154). The number of theoretical plates was calculated (*2*) to be about 2400. The ratio of the "apparent" retention volumes (*3*), $(V'_R)_H/(V'_R)_D$, was 1.80 ± 0.01; this is equal to the ratio of the vapor pressures (*4*) at 53°C, $p_D/p_H = 1.08$. This is not unexpected, since it may be shown (*3*, p. 161) for any two substances that

$$(V'_R)_1/(V'_R)_2 = \gamma_2 p_2^\circ/\gamma_1 p_1^\circ$$

where p° is the vapor pressure of the pure solute and γ is the activity coefficient of the solute in the stationary liquid phase. This result indicates that the relative vapor pressures of isotopic molecules can be measured by gas-liquid partition chromatography in other cases where the activity coefficients are expected to be equal.

Isotope effects of similar magnitude have been encountered in the gas-liquid chromatography of tritiated substances present in radiochemical amounts. As part of an investigation of the labeled products formed when organic compounds are exposed to tritium gas (*5*), benzene was irradiated by beta particles from tritium at −195°C. The products were examined using a vapor fractometer modified by addition of a small ionization chamber within the heated enclosure and in series with the thermal conductivity cell. The outputs of the thermal conductivity cell (measuring total chemical product) and of the ionization chamber (measuring tritium) were registered simultaneously on synchronized recording potentiometers.

Two of the major tritiated products were observed to have retention volumes about 5 percent and 10 percent smaller than those of cyclohexane and of methylcyclohexane, respectively. These two radiochemical products were shown to be tritiated cyclohexane and methylcyclohexane since each was removed in the same proportion (for more than 50-percent removal) as the corresponding unlabeled compound by formation of thiourea adducts (*6*). The magnitude of the isotope effect for the tritiated cyclohexane suggests that it contains an average of three tritium atoms per molecule. The presence of molecules containing smaller and larger numbers of tritium atoms is

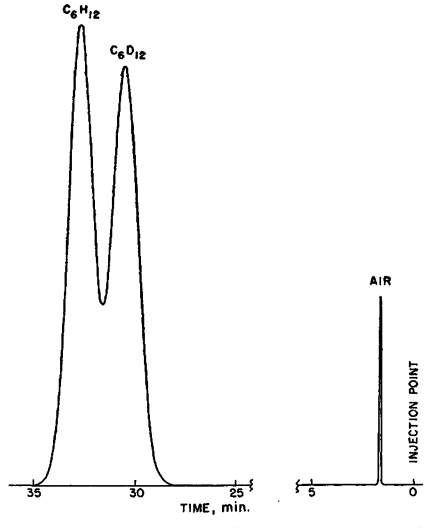

Fig. 1. Separation of cyclohexane and cyclohexane-d_{12} by gas-liquid partition chromatography.