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.

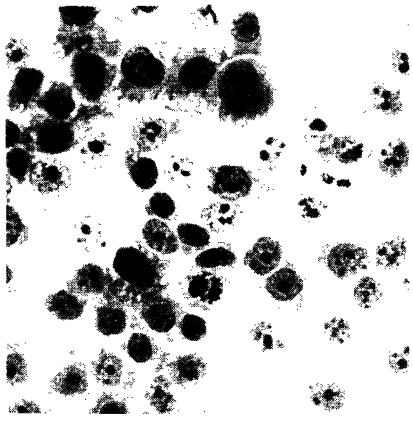

Fig. 1. Stationary culture of rabbit kidney epitheliallike cells from the fourth subculture in medium containing bovine embryo extract. Hematoxylin-eosin (× 160).

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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**References and Notes**

1. W. F. Scherer, J. T. Syverton, G. O. Gey, *J. Exptl. Med.* 97, 695 (1953).
2. This research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.
3. R. Dulbecco and M. Vogt, *ibid.* 99, 183 (1954).
4. D. Bodian, *Virology* 2, 575 (1956).
5. J. C. N. Westwood, I. A. Macpherson, D. H. J. Titmuss, *Brit. J. Exptl. Pathol.* 38, 138 (1957).
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.

31 July 1957

## 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 $1.80 \pm 0.01$; 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 $p^\circ$ is the vapor pressure of the pure solute and $\gamma$ 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

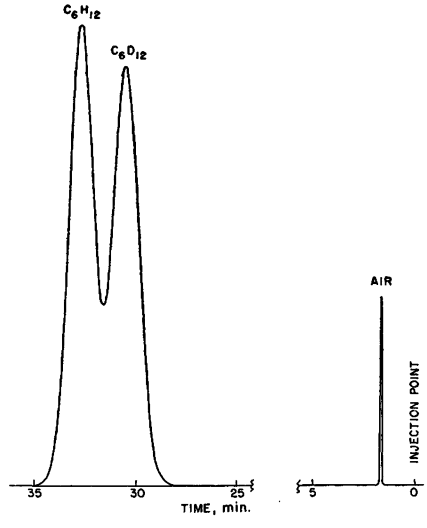


Fig. 1. Separation of cyclohexane and cyclohexane-$d_{12}$ by gas-liquid partition chromatography.

indicated by the observation that the width at half-height of the radiochemical peak is more than twice that of the corresponding chemical peak (*7*).

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**References and Notes**

1. Cyclohexane-$d_{12}$ was obtained from Merck & Co., Ltd., Montreal, Quebec. Its isotopic purity (>99 moles percent) was confirmed by mass spectrometric analysis.
2. C. Phillips, *Gas Chromatography* (Butterworths, London, 1956), p. 15.
3. A. I. M. Keulemans, *Gas Chromatography* (Reinhold, New York, 1957), p. 16.
4. R. T. Davies, Jr., and R. W. Schiessler, *J. Phys. Chem.* 57, 966 (1953).
5. K. E. Wilzbach, *J. Am. Chem Soc.* 79, 1013 (1957); P. Riesz and K. E. Wilzbach, in preparation.
6. O. Redlich *et al.*, *J. Am. Chem. Soc.* 72, 4161 (1950).
7. This research was carried out at Argonne National Laboratory under the auspices of the U.S. Atomic Energy Commission.

19 July 1957

## Sensitivity of Hamster to Colchicine

In 1952, Orsini and Pansky (*1*) reported that hamsters seem to possess a natural resistance to colchicine. They found that the hamster would survive when it was injected with dosages ranging from 0.12 to 10 mg per 100 g of body weight. Upon noting this work, we undertook an investigation to discover the lethal dosage for the hamster and to note any gross effects that might occur (*2*).

Young mature males 10 to 12 weeks of age were used for the entire series. The animals were deprived of food for 24 hours before injection but were allowed water at liberty. The weights, dosage, and subsequent history of the animals were recorded, and all animals were injected intraperitoneally in the morning.

Several animals were injected with dosages up to 10 mg/100 g of body weight, and no outward effects of the drug were noted. At the 15-mg level, slight paralysis and loss of weight were observed. The dosage was increased to 30 mg and increased by 10-mg steps thereafter. With the 30- and 40-mg dosages, all animals displayed slight paralysis in the rear quarters, drowsiness, inability to maintain equilibrium, and a marked loss of weight. The severity of these symptoms increased with increased dosages. When given 50 to 70 mg/100 g of body weight, the majority of the animals went into a coma preceded by paralysis and surges of transient tetany, from which they did not recover. One of the animals that received 50 mg and two of those that received 60 mg displayed severe nasal hemorrhages before death. No diarrhea or bloody stools were present, as has been reported for the rat (*2*). The results are recorded in Table 1.

On autopsy, pinpoint hemorrhages were present on the small and large intestines. Histological sections were made of the small intestines to observe any mitotic variation. In all cases there was a marked increase in the number of metaphase figures and an absence of spindle fibers in many cells.

Eleven males which survived the previous treatment were kept to observe any latent effects that might develop. The animals were checked, weighed, and placed with females in heat many times during the following 6 months. The males that received the 50- and 60-mg dosages never regained the tremendous weight lost, and two of the animals that received 60-mg dosages died within 3 months. Animals of these groups were hypersensitive and unsure of balance, as if their nervous or muscular system, or both, had been affected. Animals of the groups that received 30- and 40-mg dosages appeared normal and regained most or all of the weight lost.

None of the 11 males which received from 30 to 60 mg/100 g of body weight mounted a female, but all would go through the preliminary actions of breeding. However, males that received a dosage of 15 mg/100 g of body weight were fertile. Six months after the beginning of the experiment, the animals were sacrificed. In the two that had received 60-mg dosages, the following conditions were noted: the liver adhered to the diaphragm, the intestines were adhered to themselves and to the body wall, and the testes were approximately one-half of normal size. In the other groups, adhesions were not as evident and occurred only among the intestinal loops.

Histologically, the testes of these animals showed a conspicuous absence of secondary spermatocytes, spermatids, and spermatozoa. In many instances all cell types were sloughed in clumps into the lumen of the tubules. The secondary spermatocytes, spermatids, and sperm were completely absent in about one-third of the tubules of the groups that received 60 mg dosages but ranged to near normal in the group that received 30 mg dosages.

From the data given it is evident that the lethal dosage of colchicine for the hamster is approximately 70 mg/100 g of body weight. The presence of paralysis and the loss of consciousness indicate that the effects of colchicine on the nervous system are the main factor causing death. Colchicine will cause an arrest of cell mitosis in the metaphase stage in the hamster. In other work (*3*) the following is shown: (i) the effect of colchicine on the mitotic index of the crypts of Liberkühn at the 1 mg/100 g dosage level; (ii) the optimal dosage for maximal arrested metaphases; (iii) dosages that inhibit reproduction in the female; (iv) dosages that cause resorption of the fetuses in late pregnancy (*4*).

Table 1. Survival of hamsters following administration of various dosages of colchicine.

| Dosage (mg/100 g of body wt.) | No. in group | Deaths | | Survivals (No.) |
|---|---|---|---|---|
| | | No. | Time after injection (hr) | |
| 30 | 4 | | | 4 |
| 40 | 4 | 1 | 27 | 3 |
| 50 | 5 | 1 | 30 | 3 |
| | | 1 | 45 | |
| 60 | 8 | 1 | 2 | 5 |
| | | 1 | 3 | |
| | | 1 | 108 | |
| 70 | 8 | 3 | 0.5 | 0 |
| | | 2 | 2 | |
| | | 2 | 3 | |
| | | 1 | 45 | |
| 75 | 5 | 2 | 0.5 | 0 |
| | | 2 | 2 | |
| | | 1 | 3 | |

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**References and Notes**

1. M. W. Orsini and B. Pansky, *Science* 115, 88 (1952).
2. Colchicine was obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio.
3. A description of this work is in preparation.
4. This investigation was supported in part by research grant RG4473 from the National Institutes of Health, U.S. Public Health Service.

15 July 1957

## Alpha-Rhythm Responsiveness in Normal, Schizophrenic, and Brain-Damaged Persons

Routine examination of electroencephalographic records does not show that the electroencephalograms of schizophrenics differ in any consistent manner from those of normal patients (*1*). However, more active electroencephalographic techniques which introduce experimental variables in order to test for electroencephalographic changes hold more promise. Berger (*2*) long ago noted that sensory stimulation produced alpha blocking among normal persons. Later, Liberson (3) reported less reduction in alpha activity in response to light (a flash every 2 seconds) among catatonics than among psychoneurotics. Mundy-Castle (*4*) has emphasized the usefulness of more rapid photic stimulation, capable of producing alpha driving (increased amplitude or change in frequency) for