which showed that the neonatally thymectomized animal is more susceptible than the normal animal to the oncogenic activity of some chemical(16) or viral(17,18) carcinogens. They show, in addition, that genetically determined strain resistance to a carcinogen can be abolished by neonatal thymectomy. Interference with the cellular immune mechanism may thus be necessary, in some cases, to allow the full expression of a carcinogenic process. It is felt that the experimental system used here is a very convenient model with which to evaluate the role of host factors in the natural resistance to tumor growth.

Summary. Mice of the C57BL strain are normally highly resistant to the oncogenic activity of polyoma virus. Such mice were thymectomized at 3 days and injected with the virus at 4 days. Controls were sham-operated and injected with virus. Most of the thymectomized mice gained weight at the same rate as the controls and produced hemagglutination inhibiting antibodies. Pleomorphic tumors of the salivary glands developed within 2 months in 18 of 20 thymectomized mice and in only 1 of 16 controls at 4 months. It is suggested that the resistance of the C57BL strain to the oncogenic action of polyoma virus has an immunologic basis. Impairment of the maturation of immunologic faculty in mice of this strain allows the progression of antigenically distinct neoplastic clones induced by the virus.

1. Dawe, C. J., Nat. Cancer Inst. Monograph 4: *Symposia Tumor Viruses,* 1960, 67-128.
2. Habel, K., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 722.
3. Sjögren, H. O., Hellström, I., Klein, G., *Exp. Cell Res.,* 1961, v23, 204.
4. Sjögren, H. O., Hellström, I., *Virology,* 1961, v15, 214.
5. Habel, K., *J. Exp. Med.,* 1962, v115, 181.
6. Dawe, C. J., Law, L. W., Dunn, T. B., *J. Nat. Cancer Inst.,* 1959, v23, 717.
7. Law, L. W., unpublished observations.
8. Dawe, C. J., Law, L. W., *J. Nat. Cancer Inst.,* 1959, v23, 1157.
9. Dawe, C. J., personal communication.
10. Miller, J. F. A. P., *Lancet,* 1961, v2, 748.
11. Dulbecco, R., Freeman, G., *Virology,* 1959, v8, 396.
12. Miller, J. F. A. P., *Brit. J. Cancer,* 1960, v14, 93.
13. ———, *Ann. N. Y. Acad. Sci.,* 1962, v99, 340.
14. Metcalf, D., *Brit. J. Haematol.,* 1960, v6, 324.
15. Law, L. W., Dawe, C. J., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 414.
16. Miller, J. F. A. P., Grant, G., Roe, F. J. C., *Nature,* 1963, v199, 920.
17. Vandeputte, M., Denys, J., Leyton, R., De Somer, P., *Life Sciences,* 1963, v7, 475.
18. Defendi, V., Roosa, R. A., Koprowski, H., in *The Thymus in Immunobiology,* (R. A. Good, A. E. Gabrielsen, eds.), Hoeber-Harper, New York, in press.

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Osmotic and Respiratory Behavior of Surviving Rat Liver Cells.* (29238)

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An extensive literature is available on the osmotic behavior of mammalian erythrocytes [Ponder(1,2)] while the osmotic behavior of nucleated mammalian cells has been studied less extensively. Past studies have dealt with osmotic behavior of isolated cells and have been largely concerned with changes in total cell volume as a function of extracellular osmotic pressure. The present experiments were designed to study changes in nuclear and total cell volume as a function of changing effective extracellular osmotic pressure on surviving rat liver slices. In addition, changes in liver cell O_2 uptake were studied in relation to cell water content.

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Materials and methods. Male Sprague-Dawley rats weighing 150-200 g were main-

tained on a diet suggested by Griffith *et al* (3) with free access to water. The diet is balanced in respect to calories, vitamins, and minerals. The diet was used because the original experimental plan called for induction of butter yellow liver tumors in another group of rats in order to study the osmotic behavior of malignant cells in response to osmotic gradients. This part of the study had to be abandoned because lack of distinctive cell type in the tumors made measurement of cell volumes impossible by the methods employed herein.

Rats were sacrificed by decapitation and allowed to exsanguinate through the carotid vessels, the liver was removed *in toto* and placed in a petri dish over cracked ice in an ice chest. Liver slices approximately 0.5 mm in thickness and weighing between 80-140 mg were obtained with a Stadie-Riggs tissue slicer and placed in the main compartments of Warburg flasks. Each flask contained 2 ml of calcium free Krebs-Ringer Phosphate solution at pH 7.4 adjusted to 300 millosmoles (mos) with an Advanced Instruments Osmometer. One-half ml of calcium free Krebs-Ringer's Phosphate solution hypertonic to the solution in the main compartment was placed in the sidearms. These solutions had been previously adjusted by addition of NaCl so that when tipped into the main compartments the final osmolarities would be 300, 400, or 500 mos. One ml of ion free H_2O was used in the sidearms of those flasks where, after tipping, the final osmolarity was 200 mos. All solutions contained 100 mg % glucose.

Fluted filter paper strips moistened with 10% KOH were placed in the center wells. The flasks were gassed for 5 minutes wth O_2 and equilibrated in a water bath at 37.5° before the stopcocks were closed. The flasks oscillated through an arc of 45° at a rate of 120 oscillations per minute. After the first 30 minutes the apparatus was stopped and the flasks tilted to mix the contents of the sidearms with the main compartments and the O_2 uptake was then measured for the next 90 minutes, and was recorded as microliters of O_2 consumer per mg dry weight per 90 minutes. At the end of this period the tissue was removed and placed in Bouin's solution. The osmolarity of the remaining extracellular solutions was measured.

Tissues were sectioned at 5 μ and stained with hematoxylin and eosin. Liver cell nuclear diameters were measured to the nearest 0.5 μ under oil immersion, with an occular micrometer. As not all sectioned nuclei represented half spheres, frequency distributions of nuclear diameters were plotted for tissue slices at all final osmolarities. These fell into a normal distribution. Four hundred liver cell nuclei were measured at each final osmolarity in liver slices from 4 different animals. The edges of the tissue slice were avoided in measuring nuclear diameters as these contained fragmented cells, pyknotic nuclei, and staining characteristics suggesting damage during the sectioning process. The nuclear volume was calculated from the mean radius at each final osmolarity assuming the liver cell nuclei to be spherical. The mean nuclear-cytoplasmic ratio was calculated using the method of Chalkley(4) with 400 "hits" on the nuclei at each final osmolarity. The mean cytoplasmic volume (nuclear volume multiplied by the reciprocal of the nuclear cytoplasmic ratio) and the mean total cell volume (nuclear volume + cytoplasmic volume) were calculated at each final osmolarity.

Results. Data on change in nuclear diameter, nuclear volume, cytoplasmic volume, and total cell volume as a function of final extracellular osmolarity are shown in Table I.

The equation of Lucké and McCutcheon (5) compares the cell volume (V^o_{cell}) at isotonic extracellular osmotic pressure (π_0) with that cell volume (V_{cell}) at some hypertonic or hypotonic extracellular osmotic pressure (π_e):

$$\pi_e(V_{cell}-b)=\pi_o(V^o_{cell}-b) \qquad (1)$$

where b is the non-solvent volume, then:

$$V_{cell}-b=(\pi_o/\pi_e)(V^o_{cell}-b) \qquad (2)$$

and replacing $V^o_{cell}-b$ by $V^o_{H_2O}$, the isotonic cell water volume, results in:

$$V_{cell}=(\pi_o/\pi_e)V_{H_2O}+b. \qquad (3)$$

In the present study π_o and π_e were measured by the thermodynamically equivalent

TABLE I. Change in Liver Cell Volume with Change in Extracellular Osmolarity.

Final mean extracellular osmolarity (mos)	Mean nuclear diameter (μ)	Mean nuclear vol (μ^3)	A	B	C
228 (.75)	7.00 (.060)	179.6	.06597 (.0030)	2807.5	2987.1
320 (1.00)	6.41 (.104)	138.2	.06776 (.0032)	2039.7	2177.9
420 (1.75)	5.72 (.047)	98.0	.05590 (.0015)	1753.2	1851.2
513 (1.55)	5.57 (.044)	90.5	.05965 (.0018)	1517.1	1607.6

( ) Standard error of mean.

A. Nuclear/cytoplasmic ratio based on 400 random nuclear hits taken in 16 units of 25 hits.

B. Mean relative cytoplasmic volume in μ^3 (nuclear volume multiplied by reciprocal of nuclear/cytoplasmic ratio).

C. Mean total cell volume in μ^3 (nuclear volume + cytoplasmic volume).

freezing point depression (Δ^t) of the isotonic (Δ^t_0) and the final hypo- or hypertonic extracellular solution (Δ^t_1). It is clear from equation 3 that if the total liver cell and liver cell nucleus behaved as osmometers their volumes should be a linear function of Δ_0/Δ_1. Fig. 1 shows this to be the case.

O_2 uptake for the final 90 minutes in which the liver slices were suspended in extracellular solutions of varying osmolarity is recorded in Table II. Regression analysis of data relating O_2 uptake of liver slices as a function of extracellular osmolarity fails to show a significant correlation at the 95% level. However, O_2 uptake of liver slices respiring in the 228 mos extracellular solutions is significantly greater ($p<.05$) than O_2 uptake of liver slices respiring in the 513 mos extracellular solutions. Neither O_2 uptake in the hypertonic or hypotonic solutions is significantly different from that of liver slices in the isotonic solution (320 mos). In short,

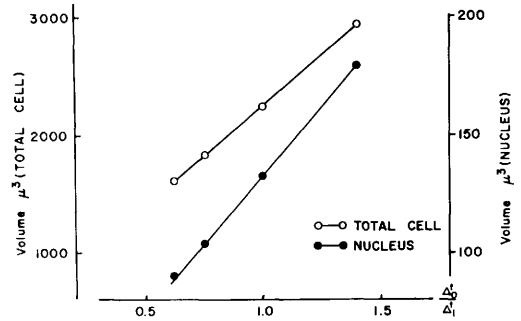

FIG. 1. Total cell and nuclear volume in μ^3 (ordinate) as a function of the ratio of freezing point depression of the extracellular fluid at isotonicity (Δ^t_0) to the final freezing point depression of the extracellular fluid (Δ^t_1) 90 min after changing extracellular osmolarity (abscissa).

a 37.2% increase or a 22.3% decrease in cell water volume does not significantly change the O_2 uptake of liver slices when compared with liver slices respiring at isotonicity.

Discussion. In vitro studies have generally shown that nucleated cells behave approximately as osmometers. Hempling(6) studying Ehrlich ascites tumor cells by densimetric methods showed that these cells followed the Boyle-van't Hoff Law in hypertonic solutions. Dick(7) using immersion refractometry demonstrated that chick heart fibroblasts behaved as predicted by the Boyle-van't Hoff equation, over extracellular osmotic ranges from 130 to 587 mos and Lucké *et al*(8) arrived at a similar conclusion for the osmotic behavior of rat lymphocytes and lymphosarcoma cells. The present study is in agreement with these findings and indicates that both the nuclear and total volume of the respiring rat liver cell behave approximately as osmometers over extracellular osmotic ranges from 218 to 513 mos. Furthermore, this cellular volume response to osmotic gradients is maintained for at least 90 minutes under the conditions of the experiment.

Isolated nuclei of amphibian oocytes have been found to be permeable to water and physiological electrolytes. [Goldstein and Harding(9) and Battin(10)]. Isolated rat liver cell nuclei are permeable to such high molecular weight substances as hemoglobin, gelatin, beef serum albumin. Nucleases [Anderson(11)], antigen [Crampton and Haurowitz(12)], and RNA [Jenner and Szarfarz (13)] have been found to penetrate intact tissue nuclei. In view of these findings and the fact that the intact rat liver cell nucleus

TABLE II. Relationship of O_2 Uptake to Water Content of Liver Cells.

Mean final extracellular osmolarity (mos)	No. of slices tested	% cell H_2O from isotonicity	90 min Qo_2 (μl/mg dry wt)	Standard error
228	23	+37.2	4.82	.40
320	16	0	4.26	.43
420	20	-14.8	4.32	.34
513	22	-22.3	3.72	.27

behaves approximately as an osmometer, it would appear that the intracellular water phase is continuous throughout the cytoplasm and nucleus; *i.e.*, the H_2O activity is equal in the cytoplasm and nucleus and the total cell H_2O responds to changes in extracellular osmotic gradients by exchange of water between the extracellular and intracellular phases. The fact that salt, water, and high molecular weight substances can exchange between the nucleus and the cytoplasm suggest that it is the semipermeable cell membrane that is ultimately responsible for nuclear volume under osmotic stress.

The effect of cell H_2O volume on cellular respiration has been studied primarily in erythrocytes. Hunter(14,15) found irreversible depression of respiration in chicken erythrocytes in markedly hypertonic media but no change in O_2 consumption of chicken erythrocytes nor in anaerobic glycolysis in beef erythrocytes upon swelling in aqueous solutions. Tipton(16) found that the respiration of erythrocytes was not influenced by wide variations of extracellular osmolarity. Olmstead(17) showed that O_2 uptake via the hexose monophosphate shunt in rabbit erythrocytes, although dependent upon substrate concentration, temperature, and pH was independent of rather large changes in cell H_2O content.

Robinson(18) studying the respiration of rat liver tissue as a function of extracellular osmolarity reported that O_2 consumption was reduced in both hypotonic (200 mos) and hypertonic (450 mos) solutions. The present study suggests that O_2 uptake is slightly reduced in hypertonic solutions and slightly increased in hypotonic solutions but more generally supports the previous work on erythrocytes indicating that O_2 consumption is largely independent of rather large variation in cell H_2O volume.

The reduction in O_2 uptake by liver slices in hypertonic solutions compared with O_2 uptake in hypotonic solutions could be accounted for by changes in cell water volumes or intracellular NaCl level. It is more probable that these differences in O_2 uptake were due to changes in cell water volume for the fact that the cells behaved as osmometers over the ranges of extracellular osmolarity reported suggest that the NaCl was effectively partitioned extracellularly.

Summary. Nuclear and total cell volume changes have been studied as a function of extracellular osmotic gradients in respiring rat liver slices. The nucleus and intact cell respond approximately as osmometers in a manner suggesting that the intracellular water activity is the same in the nucleus and cytoplasm of the cell. A mean increase of 37.2% or a mean decrease of 22.3% of cell water volume did not significantly change the cell O_2 uptake when compared with cell O_2 uptake at isotonicity although the O_2 uptake was significantly increased in cells respiring in hypertonic solutions when compared with cells respiring in hypotonic solutions.

1. Ponder, E., *Protoplasmatologia, Handbuch der Protoplasmoforschung,* Springer Verlag, Vienna, 1955.

2. ———, *Hemolysis and Related Phenomena,* Grune and Stratton, New York, 1958.

3. Griffith, A. C., Nye, N., Noda, L., Luek, J. M., *J. Biol. Chem.,* 1948, v176, 1225.

4. Chalkley, H. W., *J. Nat. Cancer Inst.,* 1943, v4, 47.

5. Lucké, B., McCutcheon, M., *Physiol. Rev.,* 1932, v12, 68.

6. Hempling, H. G., *J. Gen. Physiol.,* 1960, v44, 365.

7. Dick, D. A. T., *Proc. Roy. Soc. Lond. Ser. B.,* 1958, v149, 608.

8. Lucké, B., Hempling, H. G., Makler, J., *J. Cell. Comp. Physiol.,* 1956, v47, 107.

9. Goldstein, L., Harding, C. V., *Fed. Proc,* 1950, v9, 48.

10. Battin, W. T., *Exp. Cell. Research,* 1958, v17, 59.
11. Anderson, N. G., *Science,* 1953, v117, 517.
12. Crampton, C. F., Haurowitz, F., *ibid.,* 1950, v112, 300.
13. Jenner, R., Szarfarz, D., *Arch. Biochem.,* 1950, v26, 54.
14. Hunter, F. R., *J. Cell. and Comp. Physiol.,* 1932, v14, 412.
15. Hunter, F. R., *J. Gen. Physiol.,* 1940, v24, 297.
16. Tipton, S. R., *J. Cell. and Comp. Physiol.,* 1933, v3, 313.
17. Olmstead, E. G., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 196.
18. Robinson, J. R., *Proc. Roy. Soc. Lond. Ser. B.,* 1952, v140, 135.

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A Comparison of the Cardiac Stimulating and Bronchodilator Actions of Selected Sympathomimetic Amines. (29239)

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Early investigations by Youmans *et al*(1,2) gave results which indicate the importance of the structural groups of the epinephrine molecule for various excitatory and inhibitory actions. They proposed that there are two or more receptive mechanisms. More recent investigations(3,4) have added further emphasis to the concept of 2 distinct epinephrine-sensitive receptors and have provided additional knowledge of the importance of various structural groups for the effects obtained.

The nature of the receptor in the heart mediating cardio-acceleration has continued to defy easy classification although results obtained with the blocking agent, dichloroisoproterno(5) are in agreement with the hypothesis that these receptors are of the *beta* type which usually subserve inhibitory function in other organs innervated by the sympathetic nervous system. This concept applied to sympathomimetic amines as a class, would lead to the generalization that those which induce cardio acceleration would also cause relaxation of the smooth muscle of the bronchioles. Availability in our laboratories of a large number of structurally diverse sympathomimetic amines has permitted us to extend earlier investigations of cardiac receptor affinity and to correlate these results with action in other organs.

Material and methods. Cardiac activity was determined *in vitro* by perfusion of the isolated heart of albino rabbits(6). Inotropic activity was measured with a Grass FT .03 force displacement transducer and recorded on a Grass polygraph. Initial diastolic tension was set at 10 g at the beginning of each experiment and changes in isometric tension recorded. Chronotropic changes were determined from the above described recording. All drugs were administered directly into the aortic cannula at a constant volume of 0.1 ml and the dosage expressed in terms of the base. Unless otherwise indicated, all drugs are the racemic mixture. Each drug was tested on a fresh heart preparation at 3 or more doses. Cardiac responses were plotted on semilog graph paper and the dose producing a 20% increase in force or rate estimated from this plot.

Bronchodilator action was determined by the method of Luduena *et al*(6). Data shown in Table I include also representative values taken from published reports or from earlier experiments carried out in our laboratories and are cited in order to provide a broader comparison with the recent results obtained with the N-cyclopentyl substituted amines.

This investigation includes the following commercially available drugs, in addition to the experimental compounds shown in Table I: 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol (isoproterenol) *levo*-1-(3,4-dihydroxyphenyl-2-aminoethanol (levarterenol), 1-(3,4-dihydroxyphenyl)-2-amino-1-propanol (nordefrine), 1-(3,4-dihydroxyphenyl)-2-isopropylamino-1-butanol (isoetharine), 3,4-di-