cumarol in the chick is like that in the rat.

1. Green, J. P., Søndergaard, E., and Dam, H., in preparation.

2. Dam, H., and Søndergaard, E., *Acta Pharmacol. et Toxicol.,* 1953, v9, 131.

3. ———, *ibid.,* 1953, v9, 137.

4. Dam, H., Kruse, I., and Søndergaard, E., *Acta Physiol. Scand.,* 1951, v22, 299.

5. Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.,* 1950, v183, 123.

6. Weiner, M., Shapiro, S., Axelrod, J., Cooper, J. R., and Brodie, B. B., *J. Pharmacol. and Exp. Therap.,* 1950, v99, 409.

7. Hauser, E. P., Shafer, C. L., Corson, M., Johnson, O., Trujillo, T., and Langham, W., *Circulation,* 1951, v3, 171.

8. Brodie, B. B., Weiner, M., Burns, J. J., Simson, G., and Yale, E. K., *J. Pharmacol. and Exp. Therap.,* 1952, v106, 453.

9. Pulver, R., Montigel, C., and Exer, B., *Thrombosis and Embolism,* First International Conference, Basel, 1954, p232.

10. Dam, H., Prange, I., and Søndergaard, E., *Acta Pharmacol. et Toxicol.,* 1955, v11, 90.

11. ———, *ibid.,* 1954, v10, 58.

12. Lee, C. C., Trevoy, L. W., Spinks, J. W. T., and Jaques, L. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 151.

13. Jaques, L. B., *New England J. Med.,* 1950, v243, 395.

14. Jaques, L. B., Millar, G. J., and Spinks, J. W. T., *Schweiz. Med. Wochen.,* 1954, v84, 792.

15. Jaques, L. B., *Rév. d'Hématol.,* 1955, v10, 379.

16. Bourgain, R., Todd, M., Symons, C., Herzig, L., and Wright, I. S., *Schweiz. Med. Wochen.,* 1954, v84, 820.

17. Weiner, M., Brodie, B. B., and Burns, J. J., *Thrombosis and Embolism,* First International Conference, Basel, 1954, p181.

18. Hais, I. M., and Prochaska, Z., *ibid.,* p238.

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Increase in Induced Pulmonary Tumors in Mice Associated with Exposure To High Concentration of Oxygen. (22507)

W. E. HESTON AND A. W. PRATT.

National Cancer Institute, N. I. H., Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

Reports on the effect of concentration of oxygen upon mutation rate have been of considerable interest to those investigating relationships between mutagenesis and carcinogenesis. The original report was that of Thoday and Read(1) who observed that bean root cells X-irradiated in nitrogen contained a lower proportion of chromosomal aberrations than those irradiated in oxygen. Subsequent work(2-4) has shown that the concentration of oxygen affects the rate of gene mutations as well as that of chromosomal changes, and has also shown that the effect is not limited to irradiated cells. Increased interest in possible relationships of these observations to carcinogenesis arose when Plaine and Glass(3) reported an increase in occurrence of melanotic tumors in *Drosophila* larvae irradiated in the presence of increased concentrations of oxygen. Recently Plaine (5) has reported an increase in the melanotic tumors with increase in concentration of oxygen without radiation.

Comparison of these melanotic tumors in *Drosophila* with neoplasms in mammals morphologically is limited, but genetically similarities between the two exist. This fact together with the observations that many agents known to increase mutation rate in lower organisms are carcinogenic when tested in mice made it highly desirable to test the concentration of oxygen on the occurrence of neoplasms in mice. The fact that the effects of oxygen in the lower organisms was not limited to irradiated cells increased the possibilities for a study of the effect of concentration of oxygen on carcinogenesis. The purpose of this report is to record an increase in occurrence of pulmonary tumors in mice exposed to a high concentration of oxygen.

Materials and methods. Pulmonary tumors in the genetically highly susceptible strain A

mice were chosen for the study since in our experience they have provided a very delicate test for effects of agents upon carcinogenesis. The intravenous injection of the carcinogen dibenz[*a*,*h*]anthracene was added to the procedure in order to produce multiple tumors so that any effect could be measured on a quantitative basis and also in order to cut down the length of time necessary to keep the mice before making the observation. From former dose-response results(6) a dosage of .1 mg dibenz[*a*,*h*]anthracene dispersed in .25 cc water was selected as one which would give a response in a range of number of nodules suitable for detecting any significant change in response brought about by exposure to high concentration of oxygen. Since it was known that the crystals of the carcinogen become lodged in the capillaries of the lung it appeared that the sequence of procedures most likely to give a positive result would be to inject the animals and immediately thereafter to expose them to the high concentration of oxygen in order that any effect of the oxygen would coincide with the action of the dibenz[*a*,*h*]anthracene.

For exposure of the animals to a high ambient concentration of oxygen, a flow line was constructed which contained a flow controller, a flowrate meter, and a gas-tight exposure chamber. Oxygen gas, supplied from high pressure cylinders through a two-stage pressure reducing valve, passed sequentially through the flow controller and the flowrate meter and then into the chamber. Copper tubing, ¼ inch in diameter, was used to complete the flow line except for a small segment of tygon tubing used to connect the flowrate meter and the chamber. The copper tubing connections were made gas-tight by the use of flare-nut unions. The flow controller and flowrate meter afforded a continuously regulated and monitored gas flow of 400 cc/min. ± 1 cc into the exposure chamber. The exposure chamber was a plexiglas cylinder, sealed at the base with a plexiglas plate and closed at the top with a removable lid. The lid was pressure sealed to the body of the cylinder by means of an O-ring gasket. The volume of the chamber was approximately 2500 cc and was large enough to allow simultaneous exposure of 4 animals. A pellet food hopper and a water bottle were contained within the chamber. The animals were maintained on a wire mesh floor suspended within the chamber. The oxygen gas used in these experiments was "medical therapy" grade. Mass spectrometric analysis revealed the average gas purity for all cylinders to be 99.4% ± .3%. The contaminating gas was nitrogen. The gas inlet orifice was located at the base of the chamber so that the flow path for the gas was upward over the animals to the gas outlet orifice located in the center of the chamber lid. This flow path afforded the best possible gas mixing within the chamber volume with effective removal of the respired atmosphere thus maintaining high, relatively constant ambient levels of oxygen concentration. Samples of the effluent gas from the chamber were obtained at the end of 24 hours of the 48 hour exposure period for mass spectrometric analysis.

Observations were made on a total of 60 exposed strain A mice consisting of 40 males and 20 females with a comparable number of nonexposed controls. All animals were approximately 2 months of age when injected and exposed. There were 15 experiments each of which included 4 experimental mice all of one sex and 4 of their littermate controls of the same sex. During the course of the experiments one control was eliminated from each of 4 experiments. Both the controls and the experimental animals were injected with the carcinogen in the lateral vein of the tail and approximately 30 minutes later the experimental animals were sealed in the exposure chamber while the controls remained in the air of the animal room. The exposure lasted for 48 hours for all experiments but one in which it was extended for 96 hours. After the exposure the experimental animals were returned to the air of the animal room. Following these procedures the animals were kept in plastic cages in the animal room with 8 animals to the cage and segregated as to sex and as to controls and exposed animals. They were given Derwood pelleted food and tap water *ad libitum*. All mice were

weighed at the time they were injected; the exposed mice were weighed at the time they were removed from the chamber; and all were weighed thereafter at weekly intervals. All animals were killed and examined for tumors 4 months after the injection and exposure. The lungs were injected intratracheally with approximately 1 cc of Fekete's modification of Tellyesniczky's fixative (70 percent ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part) before the chest cavity was opened. The lungs were then removed, the lobes were separated and the tumors appearing on the surface of each lobe were counted with the aid of the dissecting microscope. Later, at least one nodule from each animal was sectioned and stained with hematoxylin and eosin for histologic examination. The eyes were routinely sectioned and stained, but no histologic changes were observed. The animals were examined for other neoplasms but none was found.

Results. The animals in the exposure chamber could be visualized at all times and the exposure procedures appeared to be tolerated well. No deaths occurred in the experimental groups during or after the exposure. Analysis of effluent gas samples obtained from the chamber showed an average oxygen depletion of 1.0%, varying for individual groups from .83% to 1.13%. The average CO_2 content was .80% varying from .71% to .91%. The average R.Q. was .84 varying from .80 to .86. The exposed mice showed a slight average reduction in weight during the exposure period but thereafter they maintained weights comparable with those of the controls.

TABLE I. Occurrence of Pulmonary Tumors in Mice 4 Months after Injection with 0.1 mg Dibenz[*a,h*]anthracene with and without Subsequent 48 Hr Exposure to Approximately 100% Oxygen. 15 experiments.

Mice exposed to oxygen			Mice kept in air		
No.	Sex	Avg No. tumors	No.	Sex	Avg No. tumors
4	♂	28.25	4	♂	10.25
4	♂	24.00	4	♂	14.25
4	♀	20.75	3	♀	6.67
4	♂	29.50	3	♂	18.00
*4	♂	85.00	4	♂	63.75
4	♂	38.50	4	♂	28.75
†4	♂	44.00	4	♂	33.00
4	♀	32.75	4	♀	19.00
4	♀	31.25	4	♀	29.75
4	♂	48.00	4	♂	24.75
‡4	♂	31.25	4	♂	26.25
4	♂	11.50	4	♂	13.50
4	♂	22.00	4	♂	16.00
4	♀	36.50	3	♀	27.33
4	♀	37.50	3	♀	28.67

* Injected with 0.2 mg DBA.
† Autopsy 4.5 mo after injection and exposure.
‡ Exposed to oxygen for 96 hr.

The results presented in Table I clearly show that high oxygen administration to strain A mice for 48 hours immediately following injection of dibenz[*a,h*]anthracene resulted in an increase in number of pulmonary tumors over that of those that only received the carcinogen. Some difference in dosage of the carcinogen resulting in some variations between experiments is apparent as may be expected with a dispersion of this kind. It is obvious that there was some increase in concentration of the dispersion as the material was taken from the container in successive experiments. There were also factors causing individual variations but these can be considered randomly distributed within each experiment. In all experiments the exposed mice had on the average more tumors than did the controls with the exception of experiment 12 in which the average number of tumors in the controls was slightly higher than that observed in the experimental animals. Among the other experiments in which the exposed animals had more tumors, 2- and 3-fold differences were observed. The probability that this 14-1 distribution could have been a chance variation from a 50-50 distribution is .0005.

Histologically these tumors appeared as adenomas typical of those that have been observed to occur spontaneously or following injection of a carcinogen. Aside from the tumors no unusual changes were noted in the tissues of the lung.

Discussion. Further study will be necessary before offering any discussion on the mechanism by which the exposure to high concentration of oxygen results in an increase in occurrence of pulmonary tumors in mice. The parallel between the effect of high concentration of oxygen on mutation rate and its

effect on induction of pulmonary tumors is of interest, but it must be suspected that agents capable of producing chromosomal changes may also produce other changes of the cell and that some of these other changes may be associated with carcinogenesis.

Data on pulmonary tumors that can be related to the present study were published by Klein(7) who was studying transplacental passage of urethan in the induction of pulmonary tumors in mice. He noted a greater number of nodules in one series of mice that were born by caesarean section and were treated differently from those in the other series in a number of other ways including being placed for a short time under a funnel through which 95% oxygen flowed. He suggested the over-all handling of the caesarean born animals might have increased dosage of urethan, or affected fetal lung metabolism or the metabolism or elimination of the urethan, but he did not report any attempt to identify which of the variables was responsible. From the observations reported herein one could assume that the increase in number of nodules he observed was due in part at least to the exposure to the oxygen.

From the standpoint of future experimentation, the importance of the demonstration of an effect of molecular oxygen on the neoplastic process can not be minimized. Caution should be taken, however, in any attempt to relate directly these observations on pulmonary tumors in mice with the problem of pulmonary cancer in man. It is well recognized that the pulmonary tumor of the mouse is not comparable to the usual lung cancer in man either in respect to its cell of origin or its usual degree of malignancy. Furthermore, a potent carcinogen, dibenz[*a,h*]anthracene, was used in the study reported herein.

Summary. Strain A mice injected at 2 months of age with dibenz[*a,h*]anthracene and immediately thereafter exposed for 48 hours to approximately 100% oxygen had when killed and examined 4 months later a significantly greater average number of pulmonary tumors than did the controls injected with dibenz[*a,h*]anthracene and kept in air.

1. Thoday, J. M., and Read, J., *Nature*, 1947, v160, 608.

2. Glass, B., and Plaine, H. L., *Proc. Nat. Acad. Sci.*, 1952, v38, 697.

3. Plaine, H. L., and Glass, B., *Cancer Research*, 1952, v12, 829.

4. Conger, A. D., and Fairchild, L. M., *Genetics*, 1951, v36, 547.

5. Plaine, H. L., *Genetics*, 1955, v40, 268.

6. Heston, W. E., and Schneiderman, M. A., *Science*, 1953, v117, 109.

7. Klein, M., *J. Nat. Cancer Inst.*, 1952, v12, 1005.

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Effect of Choline on Phosphatide Metabolism of Choline-Deficient and Cholesterol-Fed Rabbits.* (22508)

N. R. DI LUZIO† AND D. B. ZILVERSMIT.
Division of Physiology, University of Tennessee, Memphis.

It has been amply demonstrated that the administration of a single dose of choline stimulates liver phosphatide turnover in choline-deficient animals(1). These findings have been interpreted(1) as evidence that choline exerts its lipotropic effect through its action on liver phosphatides. In the present study we have investigated the effect of choline on phosphatide metabolism of choline-deficient and of cholesterol fatty livers of rabbits and have compared the action of a single dose of choline with the effects of prolonged

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