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.

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

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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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TABLE I. Phosphatide Concentrations and Specific Activities 6 Hr after P^{32}.

		High fat-low protein diet			
		No treatment	Single dose intrav. choline	Daily oral choline	Purina chow
	No. of animals	5	6	6	9
Liver	mg P/g*	1.25 ± .09	1.16 ± .12	1.17 ± .04	1.06 ± .10
	s.a.†	22.6 ± 2.05	38.7 ± 5.13	13.5 ± 1.85	12.4 ± 1.18
Plasma	mg P/g	.071± .009	.068± .007	.094± .016	.026± .002
	s.a.	10.4 ± 2.21	16.1 ± 1.39	4.7 ± 1.01	6.2 ± .90
Aorta	mg P/g	.19 ± .02	.18 ± .02	.15 ± .01	.19 ± .01
	s.a.	12.2 ± 3.14	12.2 ± 3.02	8.5 ± 1.12	7.6 ± .90

* mg phosphatide P per g fresh tissue or per ml plasma.
† Specific activity is % of injected P^{32}/g phosphatide P. Numbers following ± are stand. errors.

administration of this agent.

Methods. Albino rabbits weighing 1.5-3.1 kg were placed on Purina rabbit chow for at least 2 weeks and then were either maintained on this diet or transferred to one of the various experimental regimens. One group of 11 rabbits consumed approximately 30 g/day of a high-fat, low-protein, choline-deficient diet‡(2) for a period of 14 days. Another group of 6 rabbits were fed the same diet, to which was added 0.3 g of choline chloride§ per day. When the diet was fed in a frozen state the animals consumed it readily. Experimental atheromatosis was induced by supplementing Purina rabbit chow with 1% cholesterol§ and 2.8% vegetable fat‖ for 5 months. At the termination of the dietary period 0.5 mc of radioactive phosphate (P^{32}) was administered intravenously to experimental and control animals. Simultaneously, some of the animals were given one intravenous injection of 20 mg of choline chloride/kg of body wt as a 1% solution.¶ Six hours later, after the withdrawal of a terminal blood sample, all rabbits were killed with intracardiac Na-pentobarbital. Tissue lipids were extracted with alcohol and ethyl ether, evaporated under vacuum, and reextracted with petroleum ether. Aliquots of the petroleum ether extract were analyzed for radioactive and chemical phosphatide phosphorus(3).

Results. Table I lists the concentrations and specific activities of liver, plasma and aortic phosphatides 6 hrs after intravenous P^{32}. The liver phosphatide concentration and specific activity in the animals on the high-fat, low-protein diet which was supplemented daily with 1% choline did not differ from the corresponding values in the animals fed Purina chow. Thus it would appear that even though the high-fat, low-protein, choline-supplemented diet differed considerably from the Purina chow, a normal liver phosphatide metabolism was maintained on the synthetic diet. Removal of choline from this diet (no treatment column) resulted, however, in a 70% increase in the specific activity of the liver phosphatides. A further 70% elevation in specific activity was achieved by a single intravenous dose of choline. Thus, a single dose of intravenous or oral(1) choline appears to stimulate liver phosphatide synthesis whereas daily addition of choline to the high-fat diet seems to depress liver phosphatide synthesis below that observed in the untreated animals. The observation that chronic choline supplements, in amounts sufficient to exert lipotropic action, result in depressed liver phosphatide turnover may reflect the greatly decreased amount of fatty substrate in the livers of the supplemented animals as compared to that present in the choline-deficient group.

The plasma data follow the same pattern as those of liver, which is not surprising in

‡ Diet had the following composition: 38% lard, 44% sucrose, 8% vitamin test casein, 3% brewer's yeast, 2% cod liver oil and 5% Cowgill's salt mixture.

§ Cholesterol and choline chloride for feeding was donated by Merck and Co.

‖ The Humko Co., Memphis.

¶ Sterile choline chloride donated by the Fine Chemical Division, American Cyanamid Co.

TABLE II. Phosphatide Concentrations and Specific Activities of Cholesterol-Fed Rabbits 6 Hr after P^{32}.

		No treatment	Single dose intrav. choline
	No. of animals	4	4
Liver	mg P/g*	1.27 ± .24	1.27 ± .19
	s.a.†	17.9 ± 1.4	17.9 ± .8
Plasma	mg P/g	.233± .024	.195± .025
	s.a.	3.8 ± .61	3.7 ± .44

* mg phosphatide P per g fresh tissue or per ml of plasma.

† Specific activity is % of inj. P^{32}/g phosphatide P. Numbers following ± are stand. errors.

view of the fact that a considerable portion of the plasma phosphatides are derived from the liver(4). All animals on the high-fat diet showed a 3- to 4-fold increase in the concentration of plasma phosphatides and only small differences within the various experimental groups. This is in agreement with the observation of Bloor(5) that the administration of high-fat diets to rabbits resulted in a marked increase in plasma phosphatide, cholesterol and fatty acids. The aorta data are included in Table I because of our previous demonstration that on a high-cholesterol diet the phosphatide turnover in the thoracic aorta was markedly increased(6). The data (bottom row) indicate that the high-fat, low-protein regimen did not affect aortic phosphatide synthesis and that a single dose of choline, which had a marked stimulatory effect on liver phosphatide synthesis, did not alter the synthesis of these lipides in aorta. Apparently, the phosphatide turnover of aorta is regulated by factors other than those which influence this process in liver.

Since the cholesterol-fed rabbit is characterized by severe lipide accumulation in liver and plasma, it appeared of interest to study the effect of choline on phosphatide metabolism of this animal. The data in Table II refer to the effects of intravenous choline on the phosphatide metabolism of rabbits maintained for 5 months on a 1% cholesterol intake. Noteworthy are the markedly increased levels of plasma phosphatides to values even exceeding those of the animals on the high-fat diets. The specific activity data demonstrate that the injection of choline had no stimulatory effect on the synthesis of liver or plasma phosphatides, an indication that these animals were not choline-deficient.

Summary. In rabbits maintained on a high-fat, low-protein, choline-deficient diet for a period of 14 days, the synthesis of liver phosphatides was markedly increased. A single dose of intravenous choline was associated with a further increment in phosphatide synthesis, while chronic choline supplements depressed liver phosphatide synthesis. The plasma phosphatide concentration and synthesis were markedly elevated in all the fat-fed animals. The aortic phosphatide synthesis remained unaltered. Choline administration to cholesterol-fed rabbits did not alter the plasma or liver phosphatide metabolism.

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