fects obtained were on breathing rate. There was a pronounced decrease in breathing rate while dogs were breathing 100% oxygen.

It was stated(2) that increased air flow resistance in humans resulted in a decreased respiratory frequency. In the human studies, unlike these on dogs, the level of resistance was not sufficient to produce hypoxia. The decreased frequency of dogs breathing 100% oxygen seems to indicate that increased air flow resistance *per se* decreases breathing rate, but if severe enough to cause hypoxia, an increase in rate is initiated. The pause between expiration and inspiration in the normal dog is retained while breathing oxygen through resistances, but is lost while breathing air in an hypoxic state. Hypoxia eliminates or greatly reduces the respiratory pause.

Conclusions. 1. When tracheal air flow is so impeded as to produce pronounced changes in inspiratory and expiratory pressures, breathing rates increase, minute volumes may remain relatively uniform and tidal volumes decrease inversely with the pressure differences. 2. Two factors are involved in the accommodation to this respiratory stress: (a) Increasing carbon dioxide tensions act as primary stimuli to more forced inspiration and expiration; (b) Decreasing oxygen tensions act as stimuli to more rapid breathing by reducing the time of the expiratory phase of the breathing cycle.

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Presence of a Carcinogenic Substance in Hens' Eggs. (23470)

J. SZEPSENWOL (Introduced by Carroll A. Pfeiffer)
Department of Anatomy, University of Puerto Rico School of Medicine, San Juan, P. R.

It was shown previously that hens' eggs contain a substance that stimulates growth and sex development. Chicks maintained on a diet made up of eggs and wheat bran grew faster and became sexually mature earlier than the control chicks of the same breed and age maintained on a Purina broiler mash diet. The females became productive relatively early and the males developed very large combs, wattles and spurs. Females maintained on the above diet for over 2 years developed cancer of the ovary(1). Recently Menge, Lillie and Denton(2) reported the presence in egg yolk of a growth stimulating factor. The authors state that the latter is destroyed by heat, since ashes of yolk are without any effect upon growth. In the experiments carried out by the author(1) the eggs used in the diet were autoclaved for at least 30 minutes at 15 lb pressure. It is possible, however, that the growth-promoting factor in this case is the same as that reported by Menge and his co-workers; the moist heat of the autoclave may not be as destructive as ashing. There is also the possibility that these are 2 different substances, since the egg diet stimulated sex development in addition to growth. More work will have to be done in order to identify the growth-stimulating substance or substances in eggs. Since chicks were the only animals used in the study of the growth-stimulating action of eggs, the question arose whether animals other than birds would be affected in their growth by a diet containing eggs and to what extent such a diet might influence the development of malignant tumors. These were the bases for a new series of experiments in which mice were used. The present is a report of the first series of experiments carried out with "A" strain mice.

Methods. Sixteen mice (8 males and 8 females caged separately) were placed on a Rockland rat diet supplemented with eggs.

TABLE I. Time of Appearance, Location and Identity of Malignancies in the Experimental Mice.

Animal No.	Sex	Age in mo	Months on diet	Primary tumors	Location	Identity of tumor	Distant metastases
1	♂	9	6	1	periton.	sarcoma	abd. wall
6	"	15	12	1	lungs	adenocarcinoma	none
7	"	19	16	1	"	"	"
8	"	20	17	1	"	"	"
9	♀	20	17	1	"	"	"
10	"	21	18	1	mammary gl.	"	"
11	"	22	19	2	lungs	"	"
					ovary	"	peritoneum
12	"	22	19	3	lungs	"	none
					ovary	"	"
					mammary gl.	"	peritoneum
13	♂	22	19	2	leg	osteosarcoma	ribs
					lungs	adenocarcinoma	liver
14	"	24	21	2	lungs	"	none
					lymphoid	leukemia	
15	♀	24	21	4	lungs	adenocarcinoma	none
					ad-cort.	"	"
					ovary	sarcoma	peritoneum
					lymphoid	leukemia	
16	"	25	22	3	lungs	adenocarcinoma	none
					ovary	cystoadenoma	peritoneum
					lymphoid	leukemia	

The eggs (one or 2 fresh eggs daily) were boiled for 20 minutes. They were then split into halves and one half placed in the cage containing the males and the other in that of the females. The mice usually eat the yolk, leaving the white intact. Sixteen other mice (8 males and 8 females) from the same litters as the above were used as controls. They were maintained on a Rockland rat diet only; no eggs were given to them. In this case also the males and females were kept in separate cages. The duration of the experiment was 22 months. Most of the experimental mice died 12 to 18 months after they were put on the egg diet. The experiment was not concluded, however, until the last experimental mouse died. Of the control mice, 6 died at the age of 15 to 20 months, the remaining 10 were killed at the age of 25 months, when the last experimental mouse died. All the animals, experimental and controls, were autopsied and any diseased organs fixed, sectioned and studied microscopically.

Results were as follows: of the 16 controls, only 2 mice developed cancer. Both were females. One died at the age of 20 months, and the other was killed at the age of 25 months. In both cases an adenocarcinoma of the lungs was found. The tumor invaded both lungs, but it did not metastasize outside of the respiratory system. Of the 16 experimental mice, 12 had malignancies at the time of death. In 6 of these mice, which died 6 to 18 months after they were put on the egg diet, only one type of malignant tumor was found in each (mice nos. 1, 6, 7, 8, 9 and 10, as shown in Table I). In the other 6 mice, which lived a little longer, 2, 3, and even 4 different malignancies were found in each of them. Moreover, in the latter group distant metastases were frequent, whereas in the first group the malignancy remained localized in the organ in which it developed.

Adenocarcinoma of the lungs appears to be the most frequent malignancy in the experimental animals, but "A" strain mice are known to spontaneously produce this type of tumor. There is, however, a great difference in the rate of appearance of this malignancy between the mice on the diet supplemented with eggs and the controls. Among the latter group only 2 mice were found to have lung tumors, while of the 16 experimental animals, which were from the same litters as the above, 10 developed malignancies in the respiratory system. The lung adenocarcinomas of the

different animals were histologically similar, although they did not seem to be of the same origin. Some appeared to have developed from the bronchial epithelium, while others, without any doubt, originated from the lung parenchyma. In all of the animals with the above malignancy, the tumors were multiple; whether they were all primary or some were secondary is not known. In one case (animal 13) the lung tumor metastasized to the liver. In this case the mitotic activity of the tumor was considerably higher than in the other lung tumors. Moreover, it is interesting that this animal, in addition to its lung adenocarcinoma, was affected by a highly active osteosarcoma. A similar phenomenon was observed in other animals with multiple primary malignancies in which distant metastases of one or even of 2 of them were found. The presence of one primary malignant tumor, consequently, does not interfere with the growth and propagation of another one.

The females were all virgins, yet 2 of them developed mammary gland adenocarcinomas. These tumors were rapidly growing and one of them (animal 12) metastasized to one of the lungs and into the peritoneal cavity, close to the kidney. This animal, in addition to its mammary tumor, had a highly active primary lung adenocarcinoma and a cystadenoma of the ovary. Cysts of ovarian origin were found, in this case, in the peritoneal cavity at a distance from the ovary.

In 3 experimental animals sarcomas of different types were found. In one of them (animal 1) the malignancy, a spindle-cell sarcoma, appeared during the 6th month of the experiment. It was considered to be of peritoneal origin because of the large mass it formed in the abdominal cavity, surrounding all the viscera and invading the pancreas and part of the liver. The muscles of the abdominal wall and the subcutaneous tissue were also in part invaded by the tumor. A large mass of necrosed fat was found in this case in the peritoneal cavity and subcutaneously. It was probably caused by enzymes liberated from the pancreas, which was invaded and partially destroyed by the tumor.

In animal 13 the osteosarcoma, with its characteristic giant cells, was a rapidly growing tumor. It consisted of a relatively large mass on the left leg and many smaller nodules upon the chest wall on each side of the sternum. The former was considered as the primary and the latter as secondary tumors, but it is possible that all of them are primary growths. Histologically, no difference in structure nor in the rate of growth could be found between them. All of these tumors were continuous with the bones of the corresponding regions. As mentioned above, this animal, in addition to its sarcoma, had a lung adenocarcinoma which metastasized to the liver.

Finally, in animal 15 the picture was quite complicated. It had an adenocarcinoma of the lungs, an adenocarcinoma of the adrenal gland (the cortex), a lymphatic leukemia (characterized by very much enlarged spleen and lymph nodes, and by the infiltration of the liver, kidneys and circulatory system with lymphocytes) and a round cell sarcoma of the ovary. The latter consisted of a large mass of round cells surrounding a few degenerated follicles. Another tumor, of the size of a small cherry, was found in the lower part of the abdominal cavity of the same animal. It was identified as a mixed-cell sarcoma of ovarian origin, but it may well have developed independently from the peritoneum. The cortical adrenal adenocarcinoma, in this mouse, consisted of a large mass of anaplastic cells with no typical organization. The medulla was completely destroyed and the tumor had invaded the capsule of the kidney, but no distant metastases were found.

The ovaries were abnormal in all of the experimental females. In some of them the follicles were either in the process of degeneration or completely absent, and in 4 mice ovarian tumors were present. In animal 11 the ovary consisted of a mass of undifferentiated cells, giving the appearance of an adenocarcinoma. In this animal cysts of ovarian origin were found in the peritoneal cavity at a distance from the reproductive organ. An adenocarcinoma of the ovary was also found in animal 12, but in this case no distant metastases were present. In animal 15, as mentioned previously, a round-cell sarcoma replaced the ovary. Finally, in animal 16 the ovary con-

sisted of a small mass of undifferentiated cells and a large number of cysts. One large cyst was found in the peritoneal cavity, without any connection with the ovary. Some of these cysts were filled with clear fluid, while others contained a yellow, viscous substance. In the females with ovarian tumors the epithelial lining of the oviduct had undergone metaplasia. No damage to the testis could be found in any of the males. A similar observation was made previously in connection with chicks maintained on an egg diet(1).

The spleen and the lymph nodes were considerably enlarged in many of the experimental animals. In 3 of them, which were identified as leukemic, in addition to the large nodular spleen and the enlarged lymph nodes, the liver, kidneys and circulatory system were invaded by lymphocytes.

The liver underwent important changes in the experimental animals. Its cells very often lost their cohesion. They became vacuolated, and a great number of them were binucleated. The nuclei in the liver cells were irregular in size, and the nucleoli abnormally large. The liver picture was particularly abnormal in animals 10, 12, 14, 15 and 16, in which numerous cells were in the process of necrosis, while other groups of cells were in mitosis. Among the latter a great number of cells were binucleated or multinucleated. In some places the liver lobules consisted of a few isolated cells, while in other places no lobular structure could be recognized. It is possible that there is some relationship between the liver damage and the development of the malignant tumors, but for the time being it is too early to draw any conclusion on the subject.

Another organ which seems to be affected by the egg diet is the pancreas, but the general organization of the gland does not change.

Summary. Sixteen "A" strain mice were maintained, from the age of 3 months, on a Rockland rat diet supplemented with hard boiled eggs, one or 2 daily. Sixteen other mice, from the same litters as the above, were kept as controls. They were maintained on the same Rockland rat diet, but without eggs. Of the latter group only 2 animals developed tumors, which were in the lungs, one at 20 and the other at 25 months of age. Of the 16 experimental mice, 12 developed malignant tumors. In 6 of them the tumors in each animal were limited to a single organ, while of the other 6 mice, which died at the age of 22 to 25 months, some had 2, others 3 and one had 4 primary malignancies. Adenocarcinoma of the lung was the most frequent malignancy. It was found in 10 of the experimental animals. Next in frequency were ovarian tumors, leukemias, mammary gland adenocarcinomas, an osteosarcoma and an adrenal gland adenocarcinoma. In many of these animals distant metastases were found. In the experimental females the ovaries underwent radical changes. Four of them became cancerous. In none of the males, however, could any damage be found in the testes. It is too early to draw definitive conclusions, but there seems to be no doubt that eggs contain a substance or substances that stimulates both normal and abnormal growth.

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