Table 1. Intrinsic activities (α) and affinities (pD_2 or pA_2 values). Ach, acetylcholine; DL-allo-mu, DL-allomuscarine; DL-e/al-mu, DL-epiallomuscarine; DL-epi-mu, DL-epimuscarine; DL-mu, DL-muscarine; DL-pr-mu, DL-propyldemethylmuscarine; H fur, furtrethonium.

Substance	Rat jejunum			Frog heart		
	α	pD_2	pA_2	α	pD_2	pA_2
Ach	1	7.1		1	7.2	
H fur	1	5.9		1	5.9	
DL-mu	1	6.8		1	6.4	
DL-e/al-mu	1	5.0		1	4.5	
DL-allo-mu	1	4.4		0.4		3.7
DL-epi-mu	1	3.9		0.1		3.8
DL-pr-mu	0		4.7			5.1

partly parasympathomimetic and partly parasympatholytic (see Fig. 2, right). High doses of acetylcholine are antagonized by allomuscarine only to a certain degree, whereas allomuscarine, as such, depresses the heart beat to the same degree. Hence DL-allomuscarine is a parasympathetic drug with a dual action, or a partial agonist. Both DL-muscarine and DL-epiallomuscarine have purely parasympathomimetic action on the frog heart. The affinities, expressed as pD_2 or pA_2 values (*11*), and the intrinsic activities of the whole series are shown in Table 1.

The results establish that the affinity and intrinsic activity depend not only on the molecular structure of the drug but also on that of the receptor (*12*).

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References and Notes

1. E. J. Ariëns, *Arch. intern. pharmacodynamie* **99**, 32 (1954); ———, J. M. van Rossum, A. M. Simonis, *Arzneimittel-Forsch.* **6**, 282 (1956).
2. J. M. van Rossum and E. J. Ariëns, *Experientia* **13**, 161 (1957).
3. DL-Propyl-demethylmuscarine was generously supplied by Dr. C. H. Eugster, Organic Institute of the University of Zürich.
4. Furtrethonium was generously supplied by Smith, Kline and French, Philadelphia.
5. J. P. Long, F. P. Luduena, B. F. Tullar, A. M. Lands, *J. Pharmacol. Exptl. Therap.* **117**, 29 (1956); B. J. Ellenbroek and J. M. van Rossum, *Arch. intern. pharmacodynamie* **125**, 216 (1960).
6. The muscarine isomers were generously supplied by Dr. F. Häffliger and Dr. E. Girod of Geigy Chemical Corp., Basel, Switzerland.
7. C. H. Eugster, F. Häffliger, R. Deuss, E. Girod, *Helv. Chim. Acta* **41**, 205, 583, 705 (1958).
8. P. G. Waser, *Experientia* **14**, 356 (1958).
9. J. M. van Rossum and E. J. Ariëns, *Arch. intern. pharmacodynamie* **118**, 447 (1959).
10. J. M. van Rossum *et al.*, *Experientia*, in press.
11. H. O. Schild, *Brit. J. Pharmacol.* **4**, 277 (1949); E. J. Ariëns and J. M. van Rossum, *Arch. intern. pharmacodynamie* **110**, 275 (1957).
12. The technical assistance of Miss C. Hurkmans, Miss R. de Groot, and Miss M. Cornelissen is gratefully acknowledged. Part of this report was prepared during a stay in the department of pharmacology, Tulane University School of Medicine, New Orleans, La., on a U.S. Public Health Service grant (2G-363).

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Increased Incidence of Tumor Metastases in Female Mice

Abstract. Most of the tumor cells injected into the tail vein of mice fail to survive at the site of arrest in the lungs, but the percentage of surviving cells is higher in females than in males. The surviving cells, however, grow at a similar and constant rate in both sexes.

The take and the growth of some transplantable tumors are known to be influenced by the sex of the recipient animal, females being usually more susceptible than males (*1*). It has been shown that the incidence of metastases is higher in female than in male mice bearing chemically induced skin tumors (*2*), and higher in estrogen-treated than in control rats bearing spontaneous mammary tumors (*3*). This higher susceptibility of female animals to tumor transplantation and metastasis may be explained through one of two mechanisms: either an increased growth rate of the tumor or an increased survival of the tumor cells originally injected or disseminated through the metastatic pathways.

In previous studies, using tritiated thymidine to label the dividing cells, we investigated the fate and the growth rate of Ehrlich ascites tumor cells injected intravenously into strain CAF_1 female mice (*4*). It was found that 99 percent of the tumor cells lodging in the lungs died in the first 48 hours, and that, from the 3rd day on, the surviving tumor cells grew at a constant rate. About 40 percent of the cells were labeled by a single injection of tritiated thymidine.

In connection with this experiment, a number of male CAF_1 mice, together with the females, were given 6×10^6 Ehrlich ascites tumor cells, from male donors, by injection into the tail vein. The animals were then randomized into four groups, each group receiving a single intraperitoneal injection of tritiated thymidine (30.5 μc per animal) at 1, 48, or 240 hours after the injection of tumor cells. The animals were sacrificed 24 hours after the injection of tritiated thymidine. The lungs were weighed, and the percentage of labeled tumor cells and the uptake of tritiated thymidine were investigated by autoradiography and liquid scintillation counting (*4*).

The results are shown in Table 1. It may be seen that the weight of the lungs increases with time after the intravenous injection of tumor cells, but more so in females than in males. As the weight of the lungs in animals thus treated is correlated with the number of secondary growths (*5*), this weight discrepancy indicates a lower incidence of metastases in the males. The uptake of tritiated thymidine by the lungs, which is essentially a function of the amount of tumor tissue present in these organs (*4*), follows a similar pattern, and on the 10th day it is five times higher in females than in males. It may also be seen that, if the tritiated thymidine label is taken as a criterion of viability of a tumor cell, the great majority of the injected tumor cells fail to survive at the site of arrest in the lungs; the number of surviving cells, however, is 10 times larger in the females than in the males. After 48 hours the percentage of labeled tumor cells, which is then a function of the growth rate of the tumor, is the same in both males and females. On the 10th day after tumor injection, the percentage of labeled tumor cells is again the same in both sexes. It should be added that, in the females, the growth rate of this tumor is known to be constant from the 3rd to the 12th day after the intravenous injection (*4*).

These data indicate that, under the conditions of the experiment reported here, the increased number of metastases in female mice, as evidenced by the heavier weight of the lungs and the higher uptake of tritiated thymidine, is due to an increased survival of injected tumor cells lodging in the lungs. Once established in the lungs, the tumor cells grow at a similar and constant rate in both sexes. It is sug-

Table 1. Uptake of tritiated thymidine in the lungs of mice injected intravenously with tumor cells. All animals were injected intravenously with 6×10^6 Ehrlich ascites tumor cells and, at the indicated intervals thereafter, with 30.5 μc of tritiated thymidine intraperitoneally.

Animals		Interval between injection of tumor cells and tritiated thymidine	Mean wt. of lungs (mg)	Uptake ($\mu c \times 10^2$)	Labeled tumor cells (%)
No.	Sex				
3	M	1 hr	226	1.55	>0.1
3	F	1 hr	232	1.38	0.8
3	M	24 hr	234	1.95	>0.1
3	F	24 hr	219	2.97	10.0
3	M	48 hr	216	5.40	35.0
3	F	48 hr	275	5.73	35.0
3	M	10 day	311	6.98	40.0
3	F	10 day	591	38.15	38.0

gested that this technique may be of some value in separating the two processes—percentage of cells surviving at the site of arrest or injection and growth rate of the surviving cells—in the investigation of the various factors (*6*) known to influence the incidence of tumor metastases (*7*).

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References and Notes

1. J. J. Bittner, *Am. J. Cancer* **16,** 322 (1932); L. Gross, *Proc. Soc. Exptl. Biol. Med.* **49,** 67 (1942); C. G. Ahlstrom and N. Stormby, *Acta Pathol. Microbiol. Scand.* **43,** 21 (1958).
2. W. E. Poel, *J. Natl. Cancer Inst.* **19,** 1013 (1957).
3. W. O. Nelson, *Yale J. Biol. Med.* **17,** 217 (1944).
4. R. Baserga, W. Kisieleski, K. Halvorsen, *Cancer Research* **20,** 910 (1960).
5. R. Baserga, P. Putong, S. Tyler, W. B. Wartman, *Brit. J. Cancer,* in press.
6. S. Wood, Jr., *A.M.A. Arch. Pathol.* **66,** 550 (1958).
7. This work was performed in part under the auspices of the U.S. Atomic Energy Commission and supported in part by a grant from the American Cancer Society (Illinois Division).

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Couplet Periodic Breathing Response to High Carbon Dioxide and High and Low Oxygen

Abstract. A breathing pattern is described which is characterized by a grouping of two breaths followed by a prolonged apnea and which may involve a mechanism different from at least one type of Cheyne-Stokes breathing. The pattern can be eliminated by breathing 9 percent O_2 or 10 percent CO_2. However, the pattern frequently persists during breathing of high oxygen.

Considerable time and effort have been devoted to the study of periodic breathing since the early observations of Cheyne (*1*) and later of Stokes (*2*). The respiratory pattern named after these men is characterized by a series of breathing efforts gradually increasing in magnitude to a peak followed by a gradual decrease, each series of respiratory efforts being separated by an apneic period sometimes lasting for 60 seconds or longer. Later, Biot (*3*) published a monograph on Cheyne-Stokes breathing in which he discussed another type of respiratory irregularity, sometimes seen in meningitis, calling attention to its differences from Cheyne-Stokes breathing. Conner (*4*) has made additional observations on this breathing pattern and re-emphasized the fact that Biot's breathing is marked by complete irregularity of amplitude and duration of the apneic periods. Thus Biot's breathing is visualized as an arrhythmic variation of respiration while Cheyne-Stokes breathing is considered to be a rhythmic variation. Precision in the classification of these two types of breathing has not always been evident, for there has been some tendency to identify any deviation from "normal" as Cheyne-Stokes breathing. Recently this looseness in terminology has again been called to account (*5*).

This report concerns the possibility of the existence of another type of respiratory irregularity distinct from those previously described (*6*).

Dogs anesthetized with thiopental sodium-chloralose sometimes show a regularly grouped pattern of breathing which is characterized by two (occasionally three or more) breaths separated in time by 3 to 5 seconds, followed by a more prolonged apneic period. Frequently this breathing pattern can be induced by giving additional injections of thiopental (Fig. 1). This couplet pattern is similar to Cheyne-Stokes breathing in that it is a rhythmic variation. However, its form differs from Cheyne-Stokes breathing in several respects: there are fewer breaths per group, the apneic phases are of shorter duration, the breaths are of about the same amplitude, and the breaths are apparently not as labored as are those at the peak of the breathing phase in Cheyne-Stokes respiration. The couplet pattern is not similar to Biot's breathing, which is an arrhythmic variation. In a strict sense Biot's breathing is not truly periodic but merely respiratory events appearing in a disoriented fashion.

The couplet pattern is always eliminated by breathing 9 percent oxygen. The significant stimulation of breathing occurring with this gas mixture indicates that the chemoreceptor mechanisms are functional.

In response to breathing 10 percent CO_2 (21 percent O_2) there usually appears a stimulation which eliminates the couplet pattern. Thus it is not essential that the animal be nonresponsive to CO_2 before the respiratory variation is evident, although some depression of the respiratory center is inevitable after the administration of thiopental. On occasion, however, the couplet pattern may appear spontaneously without administration of additional thiopental. In a few cases the administration of CO_2 caused a progressive increase in the number of breaths per group and a decrease in the duration of the apneic periods. In this latter situation there is presumably a more pronounced depression of the respiratory center.

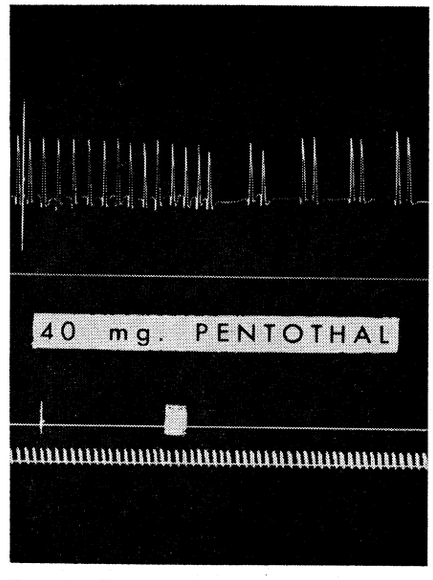

Fig. 1. Production of couplet periodic breathing by the intravenous injection of thiopental (Pentothal). The tracings (top to bottom) are as follows: pneumographic respiration, signal, and time (each tick represents 2 seconds).

In about half the cases the couplet pattern persisted during breathing of 50 to 100 percent O_2 although the apneic periods were prolonged without significant alteration of the time between the grouped breaths (Fig. 2). The various reports that high O_2 is effective in eliminating one type of Cheyne-Stokes breathing suggest that the grouped breathing here described may involve a different mechanism.

It also appears that these two respiratory patterns are not merely different stages in a progressive disruption of respiration, but rather distinct entities involving at least some differences in method of operation. This hypothesis

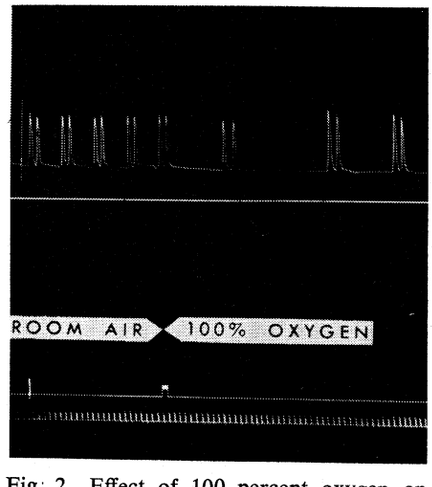

Fig. 2. Effect of 100 percent oxygen on couplet periodic breathing. Tracings are as in Fig. 1.