less than one week of treatment and therefore were not included in the study. The remaining cats showed moderate sedation and mild diarrhea but there was no indication of progressive deterioration with length of treatment nor was there any apparent correlation between these symptoms and the sensitivity to arrhythmias.

Discussion. Reserpine pretreatment causes a significant increase in the sensitivity of the cat heart to arrhythmias induced by norepinephrine and epinephrine. It seems that this sensitivity becomes progressively greater with increasing length of reserpine treatment. However, this later conclusion cannot be proven statistically with the number of experiments reported here. If the sensitivity does increase with the length of reserpine treatment, this would be quite in contrast to the positive chronotropic response of the cat heart. The increase in sensitivity of the cardiac pacemaker in the cat reaches its maximum after only 3 days of treatment with reserpine 0.1 mg/kg/day(3).

The slow onset of supersensitivity to arrhythmias in spite of 95% depletion of norepinephrine occurring within 24 hours(5) is not contrary to existing concepts. Fleming and Trendelenburg(3) have shown that the development of supersensitivity requires time following depletion and that the time required varies considerably from one response to another, supersensitivity of the nictitating membrane contraction reaching maximum much more slowly than that of chronotropic response of the heart.

The ultimate increase in sensitivity to arrhythmias seems rather great, since 2 of the 28-day reserpine cats responded to a dose of norepinephrine (0.1 μg/kg) one-thirtieth of the lowest dose causing arrhythmias in control cats. The increased sensitivity to catecholamine-induced arrhythmias is a hazard worthy of consideration in individuals on reserpine therapy.

Summary. Pretreatment with reserpine (0.1 mg/kg/day) for 7 to 28 days causes a significant increase in the sensitivity of the cat heart to catecholamine-induced arrhythmias. There is some indication that the sensitivity increases progressively with increased duration of reserpine treatment.

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Carbon Dioxide Induced Acidosis in Pregnant Rats and Caries Susceptibility of Their Progeny. (27831)

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The information regarding prenatal influences on tooth development is quite limited. Stein(1) reported 8 cases of enamel hypoplasia and Sheldon *et al.*(2) observed hypoplastic defects of intrauterine origin. The maternal influences of rickets and syphilis were noted by Wolfe(3) and by Diamond and Weinmann(4) while Kreshover(5) observed clinically that metabolic disturbances altered tooth formation. Among other studies are those of Mellanby(6) on abnormally shaped incisors and molars of rats whose mothers were vitamin A deficient during pregnancy, and Kreshover(7) showed that alloxan treatment during pregnancy in the rat produced abnormalities of amelogenesis.

There have also been a limited number of observations on prenatal influences and caries susceptibility in the offspring. Thus Sognnaes(8) fed pregnant rodents a 67% carbohydrate diet and their progeny had an increased incidence of dental caries, while Watson and Muehler(9) found that a significant decrease in dental caries incidence was obtained in animals born to mothers on a high sucrose diet. Ershoff and Bavetta(10) demonstrated that X-irradiation during certain periods of prenatal development significantly increased the severity of dental caries in rats subsequently fed a cariogenic diet. The offspring of rats administered a single dose of 150 R-X-irradiation on the 10th or 14th day of pregnancy showed a significant increase in severity of caries over that of the offspring of non-irradiated rats. They also found that the severity of caries was not increased in the offspring of rats administered a similar dose of X-irradiation on the 18th day of pregnancy.

Stanmeyer *et al.*(11) studied the effects of carbon dioxide toxicity on formation of dentin and found that the dentin matrix of the incisor formed normally under CO_2 but failed to become calcified. Concomitantly the serum calcium was elevated and the phosphorus values were low and bands of hypercalcification appeared in the incisors.

Further evidence of the effects of carbon dioxide on calcium metabolism was demonstrated by Schaefer(12) who found that, in subjects exposed for long periods of time to a relatively low concentration of carbon dioxide, there appeared to be an increase in calcium and a decrease in phosphorus in the red cells. Schaefer *et al.** exposed guinea pigs to 15% CO_2 for periods of up to 7 days with recovery periods up to 2 weeks in air, and found a significant decrease in inorganic phosphorus and an increase in calcium content of the blood serum. King(13) observed that when rats were exposed to an atmosphere of 30% CO_2 and 70% O_2, an extreme acidosis (blood pH of 6.87) is produced within 5 minutes of exposure. That the sequence of events produced under lower concentrations of CO_2 occurs much quicker under these higher conditions confirms Schaefer's† theory that there is a time-concentration relationship in exposure to carbon dioxide. Exposure of the mothers on the 10th day of gestation to increased concentrations of this gas in the breathing air, has been shown by Haring (14) to produce cardiac and skeletal malformations in the rat. Carbon dioxide and X-irradiation are both mild teratogens and both seem to have similar effects in producing metabolic disturbances. It was the purpose of this study to investigate the effect of respiratory acidosis, as produced with excess CO_2 in the air at different stages of gestation in the rat, on the caries susceptibility of the progeny.

Materials and methods. Fifty female rats of the Sprague-Dawley strain which had been raised from weaning on Purina laboratory chow were used in this experiment. Once they had reached an average body weight of 190 g, they were mated to males of proven fertility and divided into 6 groups as indicated in Table I. The period of pregnancy was timed from the morning that spermatozoa were found in the vaginal smear, and that day was considered as day 0.

The first 2 groups consisted of young whose mothers did not receive treatment; the first group was fed stock laboratory pellets and the second a cariogenic diet. The third group consisted of young whose mothers were rendered acidotic on the 7th, 8th and 9th day of gestation and their young were fed a stock diet. The procedure for rendering the pregnant rats acidotic was the same in all instances, *i.e.*, the animals were exposed to an atmosphere of 30% CO_2 in 70% O_2 for 30 minutes during the first day of treatment and for 10 minutes each during the second and third day. The blood pH remained at 6.87 during the entire period of treatment and returned to normal on recovery in air (13). The fourth group of mothers received the same treatment as Group 3 and the young were fed a cariogenic diet. In the fifth group the mothers were rendered acidotic during the 15th day of gestation, which is the beginning

* To be published.

† Personal communication.

TABLE I. Treatment to Mothers during Gestation and Caries Experience of Their Progeny.

Group	No. of rats	Treatment to mothers during gestation	Diet	Dental caries experience: % carious	Carious teeth per rat (avg)	Severity score per rat (avg)
I	50	None (control)	Pellets	0	0	0
II	67	*Idem*	635.5	83.6	2.96	9.25 ± 1.23*
III	50	30% CO_2, 70% O_2 during 7th, 8th & 9th day	Pellets	0	0	0
IV	75	*Idem*	635.5	97.4	4.20	19.25 ± 1.8
V	25	30% CO_2, 70% O_2 during 15th, 16th & 17th day	635.5	82.6	3.27	9.66 ± 1.3
VI	25	30% CO_2, 70% O_2 during 17th, 18th & 19th day	635.5	83.0	3.18	7.59 ± 1.5

* Stand. error.

of the differentiation of ameloblasts or the beginning of dentin apposition, and also on the 16th day and 17th day. Their young were fed the cariogenic diet. Finally, in the sixth group the mothers were rendered acidotic late in pregnancy, *i.e.*, during the 17th, 18th and 19th day of gestation, and their young placed on the cariogenic diet.

During the first day of life the litters were reduced to 6 to assure adequate suckling; they were weaned at 21 days of age and placed on the particular diet and tap water *ad libitum*. The cariogenic diet differed from diet 635 of McClure and Folk(15), in that 50% of the roller skim milk powder was autoclaved. This diet was numbered 635.5. They were given Vit. A, D, and E dropwise twice a week.

After 65 ± 5 days on experiment they were killed by sodium nembutal anesthesia and immediate exsanguination by cardiac puncture. Blood serum calcium was determined by Munson's(16) technic and inorganic phosphorus by the method of Chen *et al.*(17). The rats were decapitated, the skulls cleaned of all soft tissues and the lower molar teeth scored for caries as previously described(15).

Results. None of the animals, experimental or control, on stock laboratory pellets developed caries. Of the animals on the cariogenic diet alone, Table I, Group II (control), 83.6% developed caries with an average severity score of 9.25 ± 1.23. The animals from Groups V and VI, Table I, were from mothers rendered acidotic late in pregnancy and their caries picture did not vary significantly from the controls.

However the data for Group IV rats (Table I) were obtained from animals whose mothers had been rendered acidotic early in pregnancy. In these rats the following increases over the controls in the caries experience was observed: (1) caries incidence was increased from 83.6 to 97.4%, (2) total number of lower carious teeth increased from 2.96 to 4.20, and (3) the average severity score rose from 9.25 ± 1.23 (control) to 19.25 ± 1.8 in the test group.

The treatment did not alter the pattern weight gain of the young which were weighed every week. Total weight change is presented in Table II. The rats fed pellets or the cariogenic diet gained about the same regardless of the treatment to the mothers. A weight change was observed between animals on the cariogenic diet and those on pellets.

Table III presents serum calcium and inor-

TABLE II. Weight Gain 65 ± 5 Days after Weaning.

Group	Treatment	Diet	Wt gains, g
I	None	Pellets	232.0 ± 14.2*
II	"	635.5	136.6 ± 3.2
III	30% CO_2, 70% O_2 during 7th, 8th & 9th day	Pellets	263.5 ± 13.5
IV	*Idem*	635.5	158.4 ± 10.0
V	30% CO_2, 70% O_2 during 15th, 16th & 17th day	635.5	135.3 ± 15.0
VI	30% CO_2, 70% O_2 during 17th, 18th & 19th day	635.5	145.2 ± 15.0

* Stand. error.

TABLE III. Serum Calcium and Phosphorus.

Group	Treatment	Calcium, mg %	Phosphorus, mg %
I	(Control), lab chow	9.80 ±.06	8.50 ±.13*
II	("), diet 635.5	10.40 ±.18	7.27 ±.19
III	30% CO_2, 70% O_2 during 7th, 8th & 9th day and lab chow	10.17 ±.18	7.94 ±.22
IV	30% CO_2, 70% O_2 during 7th, 8th & 9th day on diet 635.5	10.30 ±.09	7.57 ±.11

* Stand. error.

ganic phosphorus data. The cariogenic diet alone increased the calcium serum level from the control value of 9.80 to 10.40 and decreased the phosphorus level from the control value of 8.51 to 7.27 mg %. Both Groups III and IV on pellets and diet plus CO_2 also demonstrated this slight hypercalcemia and hypophosphotemia.

Discussion. Ershoff and Bavetta(10) demonstrated that X-irradiation has a teratogenic action, producing mainly anophthalmia or microphthalmia when the mothers were irradiated during the 10th day of gestation. This treatment as was noted also increased significantly the severity of dental caries in offspring fed a cariogenic diet. Although our treatment with 30% carbon dioxide in 70% O_2 before the 10th day of pregnancy did not produce obvious skeletal malformations, it did significantly increase the severity of dental caries in the offspring subsequently fed a cariogenic diet. It is important to note that although both of these prenatal treatments potentiated dental caries, as developed by a cariogenic diet, the offspring did not develop caries on the regular stock diet. Thus these prenatal treatments seemingly are responsible for aggravating the actual caries process rather than increasing the caries susceptibility of the offspring as indicated by the failure of a non-cariogenic diet (lab chow) to produce caries.

Whether X-irradiation and respiratory acidosis exert their effect on tooth development *via* the same pathway or by other more subtle mechanisms is not clear. They may act simply as non-specific stressor agents or each may have a specific pathway in the metabolic processes of gestation and tooth development. Further studies on other specific and non-specific stressor agents are required.

Summary. Respiratory acidosis in the rat induced by exposure to 30% carbon dioxide in 70% oxygen previous to the 10th day of gestation significantly increased the severity of dental caries in the offspring subsequently fed a cariogenic skim milk diet. This treatment did not have any significant effect when applied after the 15th day of pregnancy.

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