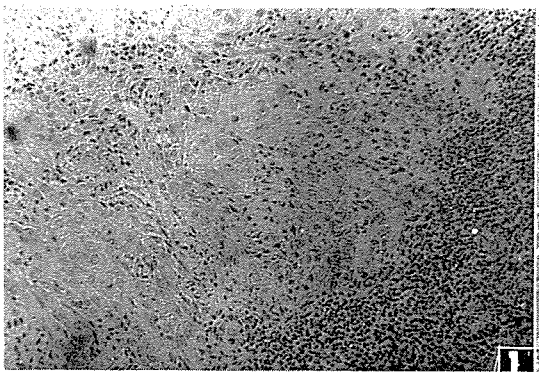
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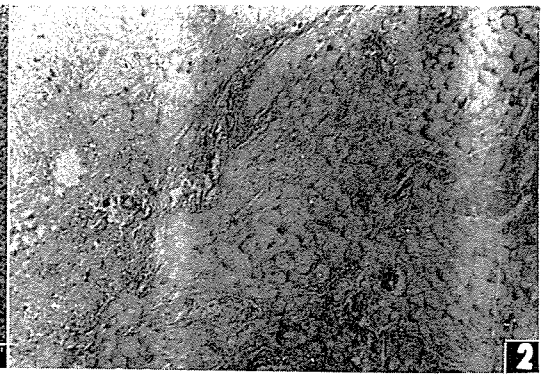
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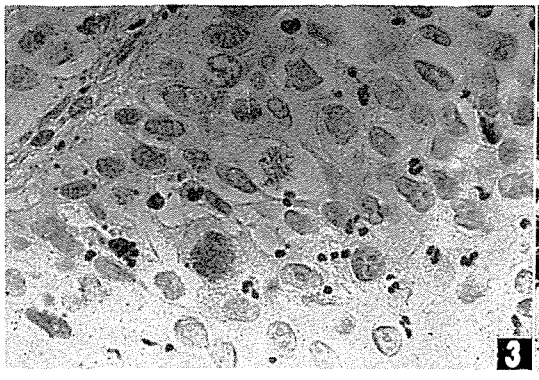
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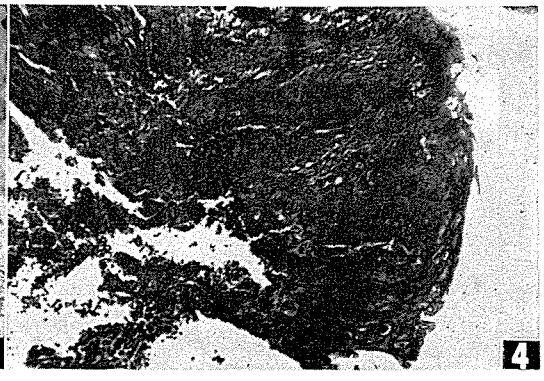
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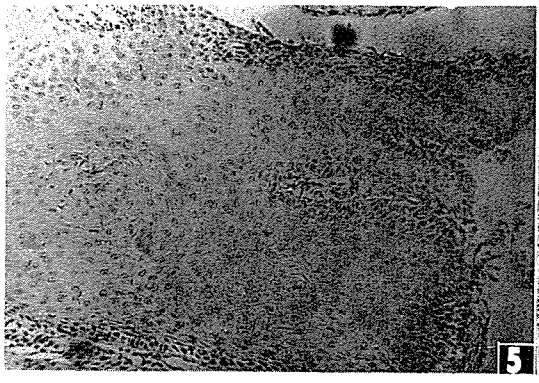
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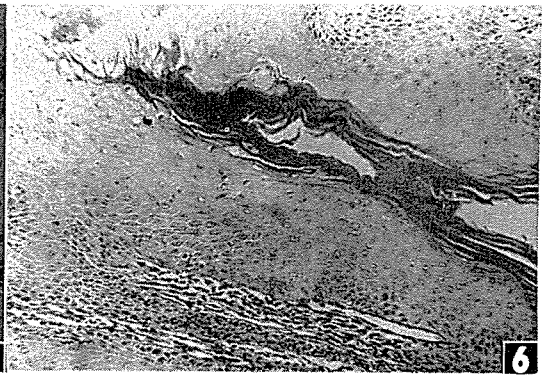
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for a period of 20 minutes in a two periods of 10 min. and 10 min. respectively. The two insults were separated by a 15 min. recovery period, by placing them in a bell jar which was flushed with a continued flow of purified nitrogen. The oxygen content in the jar, judged by use of a Westinghouse oxygen analyzer, was 30 to 40 ppm. Following the exposure to anoxia, the rats were allowed to give natural birth until day 22 of gestation. If the delivery was not made by 22 days, however, the pregnant rats were anesthetized with ether and the fetuses were removed. These subjects were regarded as being equal to those neonates which were delivered naturally. The neonatal rats were used within hours after delivery so that none of them would have experienced suckling. For each experiment, a total of 12 selected neonates were used for radioautographic preparation. These consist of 3 litter mates from the control animal, 3 litter mates each from the 3 experimental groups. representing animals insulted on days 12, 15 and 18 of fetal life. Experiments were repeated three times. All animal were maintained on purina Lab. chow and given tap water ad libitum.

Radioautographic Preparation

Each of the neonatal rats was intraperitoneally injected with DL-Leucine-4, 5-H^3* in the amount of 10 uc/gm body weight. The specific activity of the labelled amino acid was 3.90 C/mM. One animal from each of the 4 groups was sacrificed at 15, 60 or 120 minutes following the injection of leucine-H^3. The submandibular gland and the pancreas were rapidly excised, fixed for 24 hours in 10% neutral formalin and embedded in parlodion and paraplast in the standard manner. Sections 6 micra thick were made and collected on slides, coated with a subbing solution consisting of 0.5% pure gelatin and 0, 05% chromium postassium sulphate in distilled water.

Five sets of the slides coated with Kodak-NTB 3 nuclear track emulsion as described elsewhere and were exposed for varying periods. An exposure perid of 8 days turned out to be optimal for grain counts and therefore used in the quantitation of average grain numbers. Because of the poor differentiation of duct elements during the neonatal period, the grain counts were made only in definite acinar cells. The results of the grain count were processed for student t test by using an IBM 7090 computer program.

RESULTS

Since the quantitative data are recorded in Table I, Text-Fig. I and Table Ⅱ, Text-Fig. Ⅱ. the illustrative figures of radioautographs are chosen only from the tissues of the neonates that had been insulted on the 12 day of gestation and were sacrificed 120 minutes after the injection of leucine-H^3.

Figures 1 and 2 compare the pancreatic tissues of the neonates from the control and experimental animals. It is clear that the number of grains in the experimental animals is notably fewer than that of the control pancreas. Submandibular gland,

* *New England Nuclear Corp., Boston, Mass.*

depicted in Figs. 3 and 4, shows a similar tendency.

The results of grain counts from all of the experimental and control animals, given radioactive precursor at different times prior to sacrifice, are summarized in Table I, Text-Fig. I and Table Ⅱ, Text-Fig. Ⅱ. Individual data are based on pooled counts obtained from all 3 experiments. Although a significant difference between the experimental and control animals is observed in rats injected with radioactive precursor 15 minutes before the sacrifice, the difference is most pronounced in animals sacrificed at 120 minutes. In animals of this group, the average grain number of the pancreas of experimental animals varies between 50 and 75% of the control value (Table I). In all cases the difference between the experimental and control pairs is significant at the level of $p < 0.001$.

Table Ⅱ represents the quantitaive data obtained by counting cells of the submandibular glands. It may be seen that the mean grain number in control animals is only about 70% of what is found for pancreatic acinar cells at 120 minutes when the mean grain number of the controls was the greatest.

The results from the experimental animals are somewhat irregular in animals sacrificed at 15 minutes, although it is generally lower than that of the control. However, in animals sacrificed at 60 and 120 minutes after the injection of the radioactive amino acid, the number of grains per cell is the lowest in animals insulted on day 12 of gestation, reaching down to 30% or less of the control value. On the other hand, the experimental animals insulted on days 15 and 18 of gestation have a mean grain number which is twice as high as the ones exposed to hypoxia on day 12 of gestation, although they were significantly low when compared to the control. Excluding the ones that were sacrificed at 15 minutes the level of significance between the control and the experimental animals is $p < 0.001$.

DISCUSSIONS

The results described throw light upon the following three points: (1) As indicated by radioautographic quantitation following leucine-H^3 injection, the overall synthesis of proteins in normal rat neonates goes on at a greater rate in the pancreatic acinar cells than those of the submandibular gland of neonatal rats. (2) of the two glandular tissues, the submandibular gland is affected more profoundly despite the greater rate of leucine incorporation observed in the pancreas. (3) In both organs the protain synthesis was suppressed significantly in all animals insulted at different gestation periods.

Several previous workers have reported that, following exposure to anoxia, the synthesis of proteins was variously suppressed in adult organisms (Sanders, Hale and Miller, 1965; Turner and Turner, 1965). Earlier work from our own laboratory has demonstrated that similar suppressive effects were found in neonatal animals following acute anoxic exposure during the first day of life (Smith and Han, 1968; Kim and Han, 1968 and 1969). Such effects were evident in glandular cells as well as among

various types of connective tissue cells.

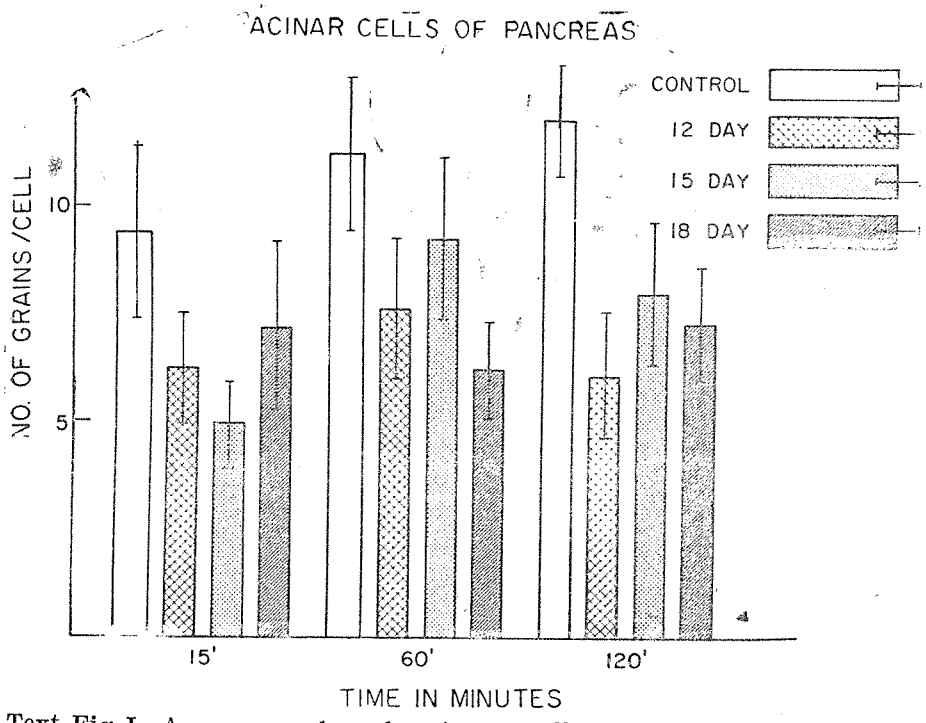
Insofar as the prenatal effects are concerned, past studies have dealt primarily with teratological aspects observable in neonates born of hypoxia-treated pregnant mother (Degenhardt, 1960; Murakami, Kameyama, and Kato, 1956; Ingals and Curley, 1957; Morawa and Han, 1968) or in chickens rendered hypoxic to hatching. These studies have clearly shown that a single prenatal residence (Grabowski, 1964) in hypoxic environment could produce profound malformations in skeletal morphology of the offspring. This was suggested to be due to the accumulation of lactic acid (Grabowski, 1964) and possible derangement of sequential evolution of enzymes and other proteins at crucial points in differentiation (Smith and Han, 1968). These statements support earlier observations of Murakami, et al., and Ingals and Curley who pointed out that the malformations were most serious in offsprings of the mothers, insulted during the time when somites were in the process of differentiation.

Except for the histological observation by Morawa and Han (1968) who suggested the possibility of a sustained impairment of protein synthesis in epidermis, few have reported any continued effects of prenatal exposure to anoxia on protein synthesis. Whether this is due to prolonged effects on rate of transcription and translation of secretory products, or due to simple retardation in degree of differentiation cannot be determined as yet, and awaits for future experimental data.

Table I. Quantitative Radioautography on Effects in Rats of Anoxia Given at Different Times of Gestation Leucine-H^3 Incorporatioin by Acinar Cells of Pancreas

Time After H^3 Injected (min)	Treatment	Mean Grain No. (S.D.)	% of Control	Significance
15	Control	9.38 (±1.99)	100	—
	12 D. Anoxia	6.23 (±1.30)	66.4	P <0.001
	15 D. Anoxia	4.93 (±1.01)	52.5	P <0.001
	18 D. Anoxia	7.19 (±1.98)	76.8	P <0.001
60	Control	11.64 (±1.80)	100	—
	12 D. Anoxia	7.64 (±1.60)	65.7	P <0.001
	15 D. Anoxia	9.26 (±1.88)	79.6	P <0.001
	18 D. Anoxia	6.24 (±1.13)	53.9	P <0.001
120	Control	12.01 (±1.32)	100	—
	12 D. Anoxia	6.09 (±1.45)	50.7	P <0.001
	15 D. Anoxia	8.01 (±1.68)	66.6	P <0.001
	18 D. Anoxia	7.31 (±1.32)	60.9	P <0.001

* Pregnant rats subjected to total anoxia for 20 minutes and immediately injected with 10 uc/gm b.w. of leucien-H^3 (specific activity: 3.90 c/mM), radioautographic exposure, 8 days.

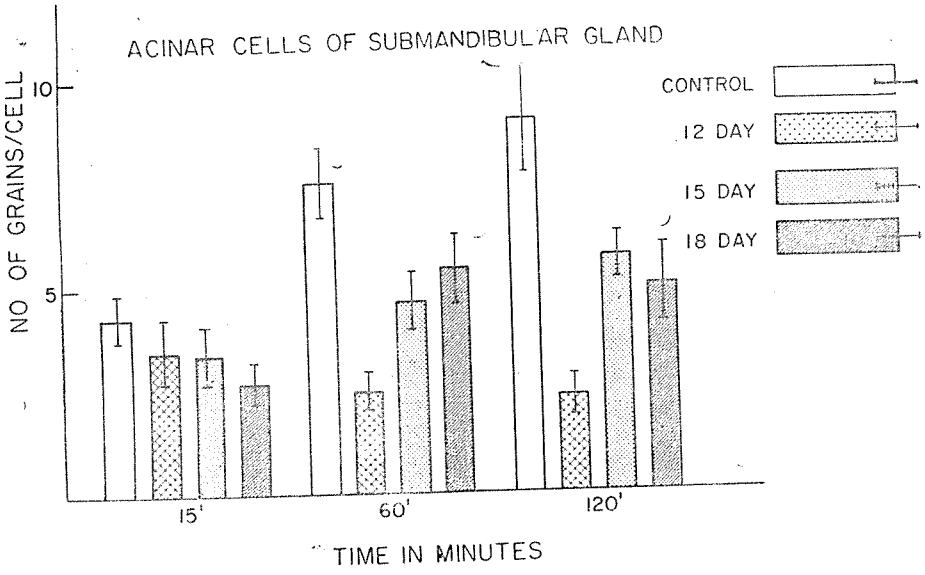


Text-Fig. I Average number of grains per cell in control and experimental acinar cells of pancr-eas at varing periods after Leucine-H^3 injection. The range indicates the standard deviation.

Table II. Quantitative Radioautography on Effects In Rats of Anoxia Given at Different Times of Gestation
Leucine-H^3 Incorporation by Acinar Cells of Submandibular Glande*

Time After H^3 Injected (min)	Treatment	Mean Grain No. (S.D.)	% of Control	Significance
15	Control	4.29 (±0.57)	100	—
	12 D. Anoxia	3.47 (±0.79)	80.9	P <0.02
	15 D. Anoxia	3.41 (±0.72)	79.5	P <0.01
	18 D. Anoxia	2.67 (±0.50)	62.2	P <0.001
60	Control	7.48 (±0.84)	100	—
	12 D. Anoxia	2.47 (±0.44)	33.0	P <0.001
	15 D. Anoxia	4.59 (±0.69)	61.4	P <0.001
	18 D. Anoxia	6.36 (±0.84)	71.7	P <0.001
120	Control	8.94 (±1.30)	100	—
	12 D. Anoxia	2.31 (±0.50)	25.8	P <0.001
	15 D. Anoxia	5.63 (±0.55)	63.0	P <0.001
	18 D. Anoxia	4.92 (±0.96)	55.0	P <0.001

* Pregnant rats subjected to total anoxia for 20 minutes and immediately injected with 10 uc/gm b.w. of leucine-H^3 (specific activity: 3.90 c/mM), radioautographic exposure, 8 days.



Text-Fig. Ⅱ

Average number of grains per cell in control and experimental acinar cells of submandibular gland.

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EXPLANATION OF FIGURES

Radioautographs of pancreas (Figs. 1 and 2) and submandibular gland (Figs. 3 and 4) of rat neonates. Rats were sacrificed at 2 hours after the injections of leucine-H^3. Pictures were focused at the levels of silver grains, and therefore the cells appear somewhat out of focus. Magnified at 630x.

Fig. 1. Pancreas of a control rat.

Fig. 2. Pancreas of a rat exposed to hypoxia on day 12 of gestation period. The number of grains over individual cells is less than that of Fig. 1.

Fig. 3. Submandibular gland of a control rat.

Fig. 4. Submandibular gland of rat exposed to hypoxia on day 12 of gestation period.