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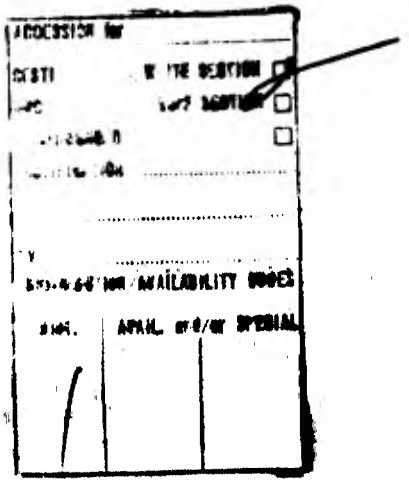

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FOREWORD

This report was prepared in the Environmental Systems Branch under task No. 793002. The work was accomplished between September 1966 and April 1967. The paper was submitted for publication on 22 June 1967.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

This report has been reviewed and is approved.

JAMES B. NUTTALL
Colonel, USAF, MC
Commander

ABSTRACT

Young male rats were maintained continuously in a hyperoxic environment for periods up to 8 weeks. During this time, the animals were subjected to an oxygen partial pressure of 369 mm. Hg (97.1%) at a simulated altitude of 380 mm. Hg (18,000 ft.). The parameters chosen to delineate growth and development in the rat were: total body weight gain; weight gain of the liver, kidney, testis, spleen, lung, adrenal, heart, and brain; deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein content of these organs; incorporation of ^{14}C-leucine into liver protein; and serum lactic dehydrogenase (LDH) isozymes. In spite of the changes reported in the text, the animals grew well in this atmosphere; however, it should be noted that several fundamental changes were observed in the growth and development on the cellular level.

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I. INTRODUCTION

Manned space travel of an extended duration is fast becoming a reality. Because of the prolonged nature of such proposed extraterrestrial flights, maintenance of a suitable environment is imperative. Choice of the atmosphere within the spacecraft remains a critical area of investigation, and much is still to be said for a one-gas, pure oxygen system. In selecting any such atmosphere, one should try to achieve a normal alveolar oxygen partial pressure. Because of the length of future space excursions, however, one may never completely discount the possibility that symptoms of oxygen toxicity may develop if the atmosphere is not properly maintained. Another problem is the lack of nitrogen in such an atmosphere and its possible deleterious effects on the individuals in the space vehicle.

Young male rats, beginning at approximately 30 days of age, were maintained in a hyperoxic environment for periods up to 8 weeks. In order to exaggerate the conditions under which symptoms of oxygen toxicity are likely to develop, an oxygen partial pressure of 369 mm. Hg was chosen. These rats were taken to a simulated altitude of 380 mm. Hg (18,000 ft.) and lived in a near complete lack of nitrogen during this period.

In the past, normal growth in the rat has been investigated by measuring weight gain of the whole body and also of the individual organs and tissues (11). In some instances this may be misleading, as in fat deposition in which there is an increase in body weight but no increase in cell proliferation. Consequently, in recent years various investigators have employed new variables for measuring the growth rate of the postnatal rat (12, 16, 35).

An increase in the weight of an organ or tissue may be the result of an increase in the number of cells (hyperplasia) or in the size of the cells (hypertrophy). On the basis of the premise that there is a constant amount of deoxyribonucleic acid (DNA) per diploid nucleus (21, 29), one can estimate the number of cells in a particular organ if the total DNA content of that organ is known. With the assumption that there is one nucleus per cell, additional information may be obtained concerning the size of the constituent cells of an organ if the total weight of that organ is known.

Protein is the major nonaqueous component of most cells. A determination of the total protein content of an organ has been employed as an index of weight gain which is not due to an increase in the number of cells (12, 20). As a reliable index of the rate of such an increase, the rate of incorporation of a labeled amino acid into liver protein has been used (25, 33).

It has been shown that the maximum rate of ribonucleic acid (RNA) synthesis occurs before the maximum rate of protein synthesis in the developing rat liver (23). Furthermore, glandular organs such as pancreas, liver, and intestinal mucosa, which synthesize large amounts of protein, are rich in RNA (8, 23). This is also true for such organs as spleen, thymus, and testis in which mitoses are frequent (8, 23). On the other hand, tissues which divide less frequently (brain, heart, and skeletal muscle) have much lower concentrations of RNA (8). Thus, determination of the concentration of RNA in a particular organ will yield information concerning the capability of that organ for synthesizing protein.

Finally, modifications of cellular enzyme systems have been shown to occur during

development and aging (5). Many studies have shown that certain enzymes possess molecular heterogeneity, and the most thoroughly studied system of this type is lactic dehydrogenase (LDH). This enzyme has been separated into five subunits or isozymes which are various tetrameric combinations of two basic subunits (19). Changes in an organ during growth and development result in a corresponding alteration of its characteristic isozyme pattern (13). Recently, this has been shown to occur in the tissues of the neonatal rat (6). Whether such tissue alterations during development are reflected in changes in the serum isozyme pattern is one of the aims of the experiment to be described.

Changes that might be detected in the parameters just described will reflect modifications in the cell proliferating mechanisms and in the protein synthesizing systems of the various organs during growth and development. Such alterations should lead to a clearer understanding of the fundamental changes involved in growth and development of the rat in the experimental environment.

II. EXPERIMENTAL PROCEDURE

White male rats of the Charles River CD* strain were used in this experiment. Both experimental and control rats were obtained at 24 ± 2 days of age and were maintained at ground level for a 7-day conditioning period. They were housed in galvanized steel cages, 2 animals per cage, and were fed Purina laboratory chow and permitted water ad libitum. Feeding, watering, and changing of the bedding were performed every day except Saturdays.

The experimental animals were maintained in the USAFSAM two-man low-pressure chamber, which has automatic environmental controls. This chamber had an internal volume of 10.76 $m.^3$ with a pass lock of 5.38 $m.^3$ The control rats were kept in a rectangular chamber (31), which also possessed a pass lock and automatic environmental controls. The environmental conditions for each chamber during the 8-week experiments are summarized in table I.

TABLE I

Environmental conditions

	Control (mean ± S. D.)	Experimental (mean ± S. D.)
Total pressure (mm. Hg)	747.1 ± 4.1	379.9 ± .3
Oxygen partial pressure (mm. Hg)	150.0 ± 7.5	368.9 ± 2.8
Carbon dioxide partial pressure (mm. Hg)	2.54 ± .98	.43 ± .49
Nitrogen (mm. Hg)	576.8 ± 13.1	.91 ± .61
Relative humidity (%)	72.7 ± 4.6	45.7 ± 9.0
Temperature (°C.)	23.5 ± .2	23.9 ± .4

Ten rats, randomly selected, were sacrificed initially (just before exposure of the remaining animals to the experimental and control environments), and after 2, 4, 6, and 8 weeks of exposure. Six of the 10 rats were employed for the following measurements: total body weight, organ weights, nucleic acid and protein analysis of the previously mentioned organs, total LDH and its isozymes, and adrenal corticosterone. The remaining 4 rats were used for the measurement of total body weight, liver weight, and radioisotope uptake studies.

Sacrifice, blood sampling, necropsy, organ weighing, and radioisotope uptake studies were performed in the pass lock in the experimental and control atmospheres, respectively. The animals were sacrificed by decapitation and the blood obtained by exsanguination. The adrenal, kidney, liver, testis, spleen, lung, heart, and brain were quickly removed, chilled in a crushed ice slurry, blotted on gauze, rapidly weighed, and placed in a jar packed in crushed ice. After exit from the lock, the organs were stored at −20° C. until analysis. After centrifugation, the serum samples were stored at 4° C. for LDH determinations the next day. The chilled livers containing the radioisotope were taken to the laboratory and processed on the day of sacrifice.

Before the nucleic acid and protein analysis, an entire organ or a 1-gm. aliquot of it was

homogenized at 4° C. with distilled water. For the heart and lung, a 10% homogenate was made using a Sorvall Omnimixer. With brain, liver, kidney, spleen, and testis, a 20% homogenate was made employing a glass-Teflon homogenizer. For adrenal analysis, six right adrenals were pooled and homogenized in 2.2 ml. of distilled water. Duplicate, 1-ml. portions of each homogenate were taken for the determination of DNA, RNA, and protein by the method of Schmidt and Thannhauser (28) as modified by Wannemacher et al. (33). This procedure is presented as a flow sheet in figure 1. Total serum LDH activity

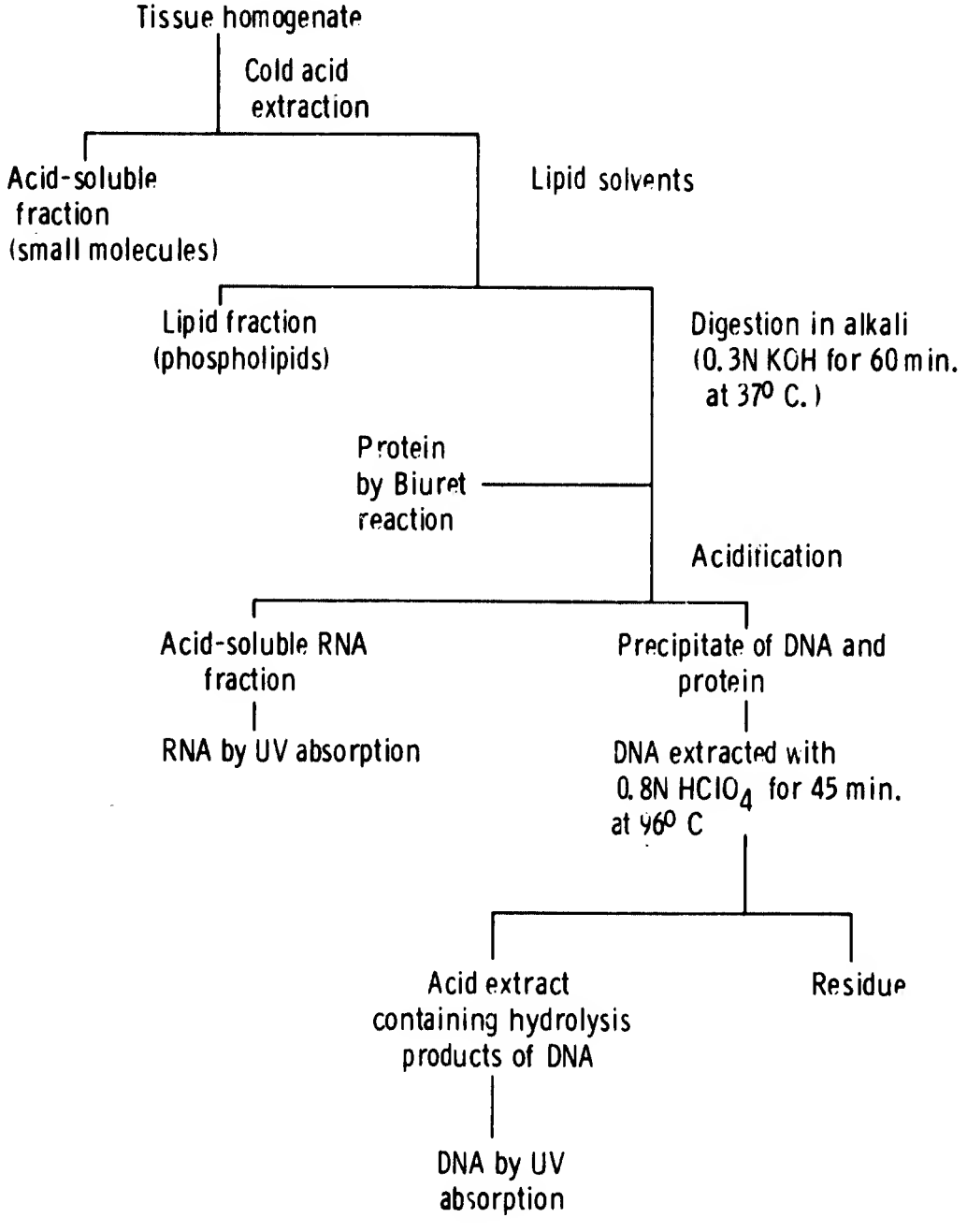

FIGURE 1

Outline of the procedure for the determination of DNA, RNA, and protein in animal tissues.

was determined at 27° C. according to the method of Bowers (7). The disc gel electrophoretic technic of Davis (9) was employed for the separation of the LDH isozymes. After electrophoresis at 2.5 ma. per sample tube for 70 minutes, the gels were placed in the reaction mixture of Goldberg and Cather (14) and incubated in the dark for 2 hours at 37.5° C. The gels were read in a Photovolt automatic densitometer with electronic integrator. The method of Zenker and Bernstein (36) was employed for the determination of adrenal corticosterone.

The amount of ^{14}C-leucine incorporated into liver protein was determined on 4 of the rats while in their respective environments. The uniformly labeled L-leucine (New England Nuclear Corp.), having a specific activity of 231 mc./mM., was diluted to 1 μc./ml. with sterile isotonic saline before injection. Thirty minutes before sacrifice, the rat was injected intraperitoneally with this solution at 1 μc./100 gm. body weight. The animal was sacrificed by decapitation, and the liver quickly removed and placed in a crushed ice slurry. A 10% homogenate in cold tris buffer, pH 7.4, was made. Duplicate 1-ml. aliquots were taken and the radioactivity in the liver protein was determined according to the method of Nirenberg (22). The samples were counted in a thin-window, gas-flow Geiger counter.

III. RESULTS

Of the 60 young male rats placed in the hyperoxic environment, none died from symptoms of oxygen toxicity during the 8 weeks of exposure. After 5 weeks of exposure, a rat with a badly festered eye was removed from the chamber and taken to Veterinary Pathology for examination. Results indicated that the injury may have occurred during a fight with its cage partner.

One week prior to beginning the experiment the control rats had an average body weight of 45.6 gm., whereas the rats to be in the hyperoxic environment averaged 59.1 gm. As shown in table II, the rats in the experimental, hyperoxic environment grew slightly faster than did the control rats during the first 2 weeks of the experiment; however, during the next 4 weeks, the control animals grew more rapidly than did the experimental rats. During the remaining 2 weeks, both control and experimental animals grew at approximately the same rate. During the entire 8 weeks of the experiment, the control animals increased their weights by 443%, while the altitude group showed an average increase of 391%.

TABLE II

Actual weight gain of young rats in the control and experimental environments

Week of experiment	Weight of controls (gm.)		Weight of experimentals (gm.)	
	Mean*	S.D.	Mean*	S.D.
−1	45.6	2.6	59.1	7.2
0	85.1	6.0	112.8	10.6
+2	166.0	28.3	223.7	11.4
+4	257.0	30.4	316.8	39.1
+6	326.1	41.6	379.6	47.7
+8	377.1	34.1	441.5	52.1

*Each value is the mean of 10 rats.

The data on the organ weights are presented in figures 2 and 3 in terms of weight per 100 gm. body weight, while the actual organ weights may be found in table III. Body weight in both groups of rats increased at a faster rate than did the organ weights. Total organ weights (table III), in all cases except the spleen, were higher for the experimental group. When these values were put on a unit body weight basis, however, the opposite was true for the spleen, testis, lung, brain, and liver. When kidney, heart, lung, brain, and liver of each group were compared on a unit body weight basis, there were only negligible differences. The spleens of the experimental animals were considerably lower than those of the control animals during the entire 8 weeks of the experiment (fig. 2). During the first 4 weeks of the experiment, the testes of the oxygen-exposed rats increased at a slightly faster rate than did their bodies (fig. 2). During the latter half of the experiment, the

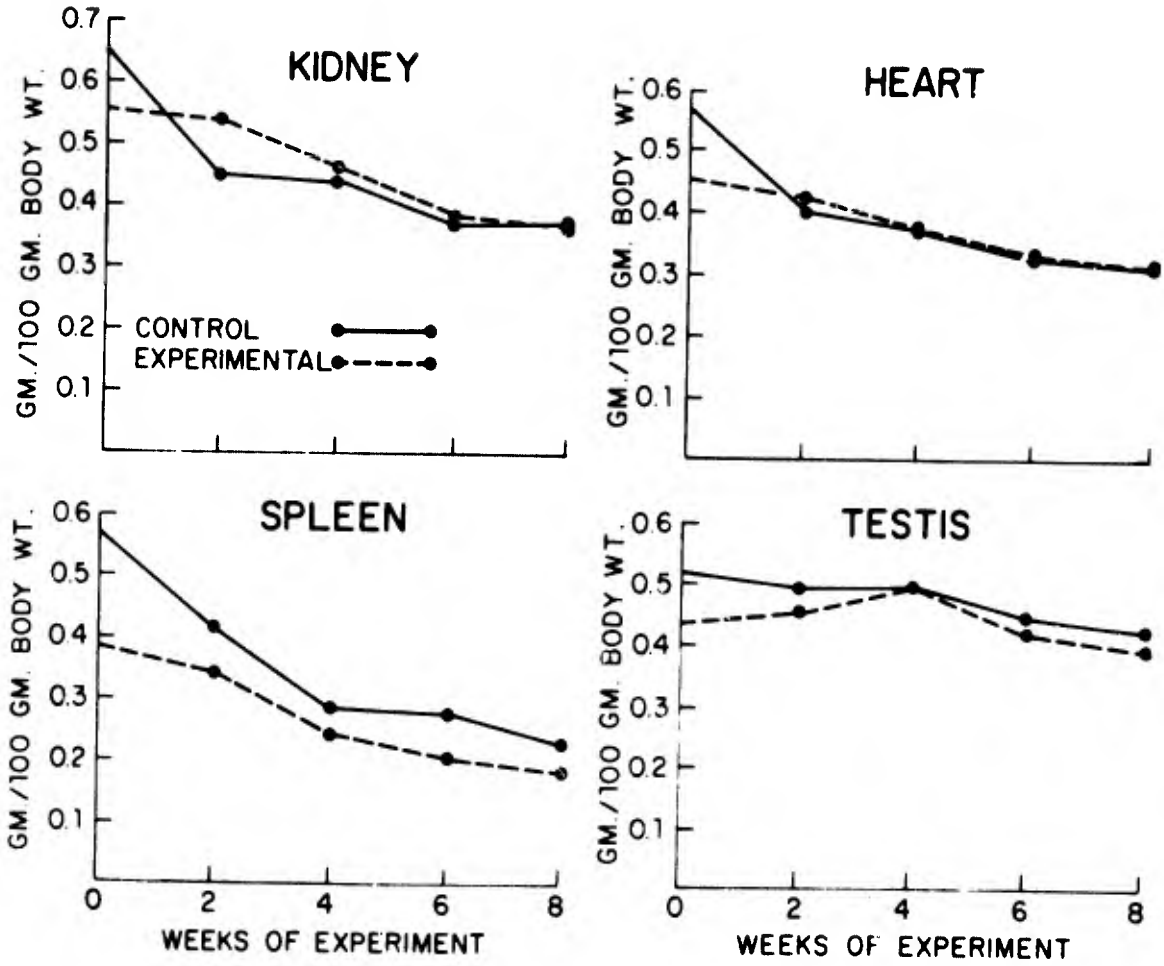

FIGURE 2

Organ weights per 100 gm. body weight in the control and experimental animals.

adrenals of the experimental rats were decreasing in weight per unit body weight at a slower rate than were those of the control animals (fig. 3).

The data on the nucleic acid and protein analysis of the eight organs are presented in tables IV through XI. Total DNA content of the livers of both control and experimental animals continued to increase during the 8 weeks of the experiment (table IV). Total protein content also increased in both groups but showed signs of leveling off in the experimental animals. An increase in organ weight without an increase in total protein might be attributed to fat deposition or an increase in interstitial fluid. A pattern of growth, similar to that in liver, was found in the testes of both the control and experimental animals (table V).

An increase in total DNA content was evident in the heart and adrenal of the controls but only during the first 6 weeks of the experiment (tables VI and VII); however, these two organs in the oxygen-exposed animals continued to increase in weight by net cell division during the entire 8 weeks. Total protein content increased throughout the 8 weeks of the experiment in both groups of animals. Net cell division or "true" growth continued in the kidneys of the oxygen-exposed rats during the 8 weeks of the experiment (table VIII). Such

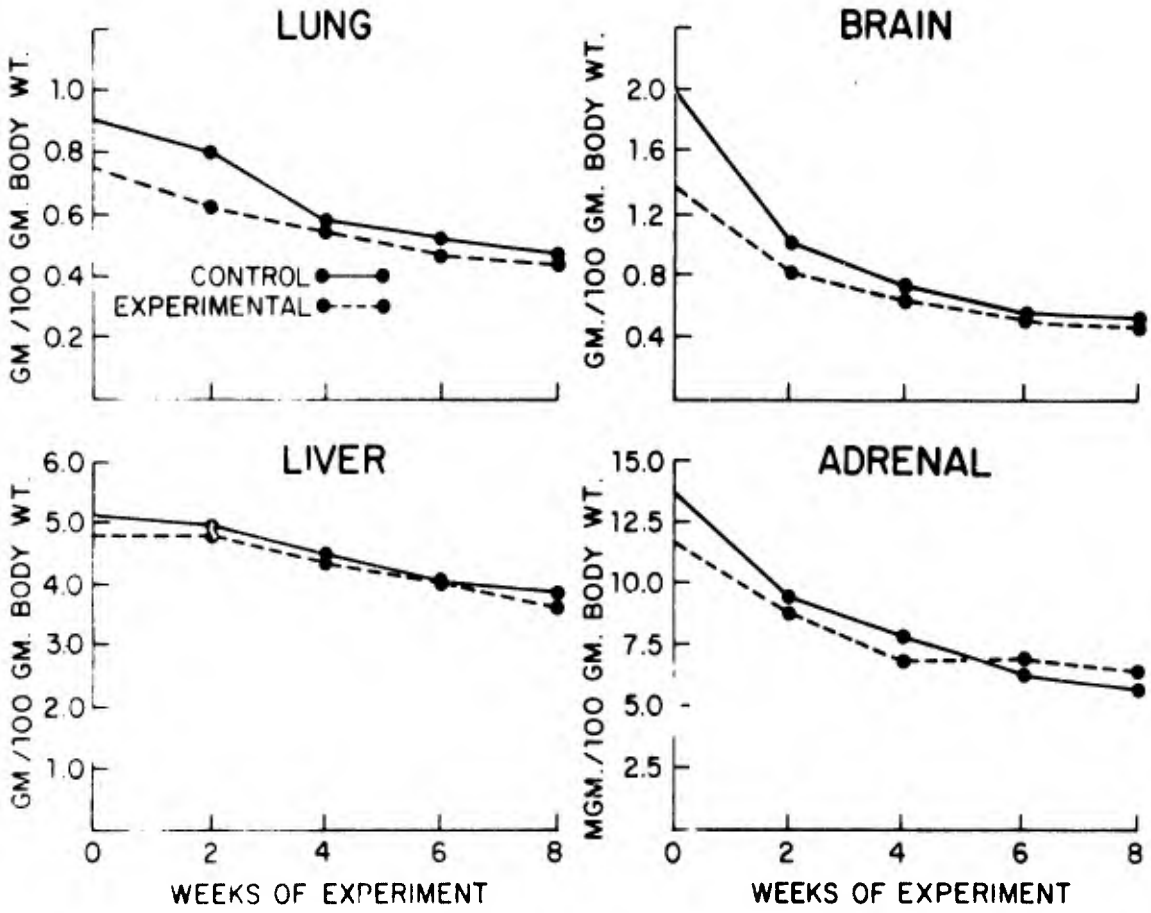

FIGURE 3

Organ weights per 100 gm. body weight in the control and experimental animals.

a trend with the kidneys of the control rats was not evident; hence, the results with this group were more difficult to interpret.

The values for lung DNA content indicate that net cell division has stopped during the middle of the experiment for both groups (table IX). Total protein content has also leveled off for both groups during this period, indicating that organ enlargement during the latter half of the experiment may be due to fat deposition or an increase in water content. The spleens of the control rats continued to grow by an increase in cell number during the first 6 weeks of the experiment, but in the oxygen-exposed animals, net cell division had stopped after the first 2 weeks (table X). This pattern was almost duplicated in the total protein content of the spleen in both groups of animals. In the brains of the control animals, total DNA content was constant during the entire 8 weeks of the experiment (table XI), indicating that net cell division had stopped before the experiment had begun. This was not quite as evident with the experimental animals. Total protein content in both groups remained essentially constant during the latter half of the experiment.

Both the control and experimental animals incorporated ^{14}C-leucine into liver protein at approximately the same rate during the first

TABLE III

Total organ weights in control and experimental rats*

Week of experiment	Animals	Kidney	Heart	Brain	Lung	Liver	Spleen	Testis	Adrenal†
0	Control	0.55	0.48	1.68	0.77	4.31	0.48	0.44	11.6
	Experimental	0.62	0.51	1.55	0.85	5.45	0.43	0.49	13.2
2	Control	0.75	0.67	1.75	1.33	8.23	0.69	0.82	15.8
	Experimental	1.20	0.95	1.83	1.40	10.72	0.77	1.01	19.3
4	Control	1.13	0.96	1.87	1.50	11.44	0.73	1.27	20.4
	Experimental	1.46	1.19	1.98	1.77	13.82	0.77	1.57	21.2
6	Control	1.20	1.06	1.87	1.71	13.11	0.91	1.45	20.[illegible]
	Experimental	1.46	1.26	1.96	1.78	15.32	0.77	1.59	26.3
8	Control	1.41	1.19	1.94	1.77	14.50	0.87	1.60	21.4
	Experimental	1.63	1.40	2.06	1.93	16.00	0.80	1.74	28.0

*Each value is the mean obtained from 6 rats.

†Adrenal weights are in milligrams, while the other organ weights are in grams.

2 weeks of the experiment (table XII). There was, however, an upward trend, possibly stimulatory, in the experimental rats during the remaining 6 weeks.

Total LDH in the serum of the controls showed an upward trend during the last 6 weeks of the experiment, which did not occur in the experimental group (table XIII). With the exception of an increase in LDH-1 in both groups, the other four isozymes in either group of animals did not display an upward or a downward trend during the experiment. LDH-3 did not occur to a measurable degree in the serums of the control rats.

Although the results on the adrenal corticosterone of the experimental rats were incomplete, the remaining values were not appreciably different from those of the controls (table XIV). Because the initial values for the two groups differed considerably, a valid comparison of the remaining values could not be made.

IV. DISCUSSION

Although none of the experimental animals died from symptoms of oxygen toxicity, such symptoms in the lung were quite pronounced in all the rats sacrificed during the 8 weeks in the hyperoxic environment. At 2 weeks of exposure a large portion of the lung (40% to 60%) was hemorrhagic and contained edema-filled alveoli. By 4 weeks of exposure, the entire lung appeared this way; however, the rats appeared to grow normally throughout the entire 8 weeks.

Mortalities as high as 30% were found in rats of the Wistar strain exposed for extended periods of time to a pure oxygen atmosphere at 258 mm. Hg total pressure (15); however, exposure of Sprague-Dawley rats to this environment for 235 days did not produce this increased mortality (15). It has been determined that there is a strain difference in their resistance to increased oxygen partial pressures at decreased barometric pressure (27). The growth rate of the oxygen-exposed rats in the present study was essentially identical to

TABLE IV

Protein and nucleic acid content of the liver in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	174.6	752.6 ± 45.7*	11.49	49.52 ± 3.19	3.57	15.38 ± 1.12
	Experimental	149.9	816.8 ± 43.4	12.50	68.12 ± 3.76	2.92	15.91 ± 1.52
2	Control	171.9	1,415.0 ± 46.0	8.24	67.81 ± 3.19	3.02	24.85 ± 2.30
	Experimental	165.6	1,774.9 ± 118.0	9.98	106.98 ± 2.35	2.63	28.19 ± 1.50
4	Control	181.6	2,077.8 ± 68.1	9.46	108.22 ± 4.80	2.46	28.14 ± 2.97
	Experimental	156.2	2,158.7 ± 65.5	12.17	168.18 ± 8.29	2.37	32.75 ± 3.86
6	Control	175.8	2,305.1 ± 97.0	9.91	129.92 ± 8.52	2.38	31.20 ± 2.62
	Experimental	173.5	2,657.1 ± 80.5	11.29	172.96 ± 9.80	2.37	36.30 ± 4.74
8	Control	178.1	2,582.4 ± 140.0	9.59	139.05 ± 10.29	2.52	36.54 ± 3.77
	Experimental	159.1	2,545.4 ± 95.0	9.33	149.28 ± 9.91	2.68	42.88 ± 2.08

*Standard deviation.

TABLE V

Protein and nucleic acid content of the testis in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	74.12	32.6 ± 3.7*	4.58	2.01 ± .20	4.47	1.97 ± .26
	Experimental	69.15	33.9 ± 3.9	5.96	2.92 ± .73	4.34	2.12 ± .27
2	Control	79.60	65.3 ± 2.3	4.92	4.03 ± .32	3.88	3.18 ± .16
	Experimental	77.86	78.6 ± 3.7	5.61	5.66 ± .42	3.58	3.61 ± .15
4	Control	77.04	97.8 ± 4.5	4.56	5.79 ± .25	2.87	3.64 ± .30
	Experimental	74.54	117.0 ± 4.7	5.57	8.74 ± .58	3.61	5.67 ± .52
6	Control	78.68	114.1 ± 4.6	4.69	6.80 ± .32	3.04	4.41 ± .24
	Experimental	79.82	126.9 ± 4.0	5.65	8.98 ± .36	3.05	4.85 ± .27
8	Control	75.04	120.1 ± 5.9	4.64	7.42 ± .38	3.22	5.15 ± .27
	Experimental	75.07	130.6 ± 9.5	4.06	7.06 ± .57	3.17	5.51 ± .56

*Standard deviation.

TABLE VI

Protein and nucleic acid content of the heart in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	132.0	63.4 ± 7.7*	2.98	1.43 ± .15	2.20	1.05 ± .13
	Experimental	127.9	65.2 ± 13.1	5.45	2.78 ± .47	2.20	1.12 ± .20
2	Control	154.6	103.6 ± 12.7	2.92	1.95 ± .37	2.23	1.49 ± .30
	Experimental	155.6	147.8 ± 19.2	2.93	2.78 ± .35	1.89	1.80 ± .30
4	Control	160.0	153.6 ± 15.0	2.76	3.39 ± .38	1.67	1.60 ± .13
	Experimental	142.9	170.0 ± 24.3	3.08	3.66 ± .85	1.67	1.99 ± .32
6	Control	144.7	153.4 ± 14.7	2.47	2.62 ± .38	1.62	1.72 ± .21
	Experimental	157.1	197.9 ± 27.6	2.82	3.55 ± .53	1.62	2.04 ± .29
8	Control	157.3	187.2 ± 22.9	2.45	2.91 ± .36	1.43	1.70 ± .26
	Experimental	160.8	225.1 ± 7.7	1.86	2.60 ± .19	1.65	2.31 ± .22

*Standard deviation.

TABLE VII

Protein and nucleic acid content of the adrenal in the young male rat*

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	127.7	1.48	8.12	0.09	6.65	0.08
	Experimental	85.9	1.12	11.55	0.15	3.33	0.04
2	Control	157.4	2.49	5.61	0.09	4.94	0.08
	Experimental	114.5	2.18	—	—	—	—
4	Control	116.2	2.37	8.24	0.17	4.31	0.09
	Experimental	94.5	1.98	6.54	0.14	2.96	0.06
6	Control	124.4	2.50	6.91	0.14	5.03	0.10
	Experimental	104.5	2.72	6.48	0.17	3.55	0.09
8	Control	123.7	2.65	7.06	0.15	4.94	0.10
	Experimental	129.9	3.64	6.51	0.18	5.17	0.14

*Six left adrenals were pooled before analysis.

TABLE VIII

Protein and nucleic acid content of the kidney in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	118.1	64.9 ± 3.2*	5.22	2.87 ± .31	5.03	2.77 ± .29
	Experimental	116.7	72.4 ± 10.1	9.39	5.82 ± .99	2.56	1.59 ± .41
2	Control	133.7	100.3 ± 6.9	5.25	3.94 ± .31	4.93	3.70 ± .25
	Experimental	117.0	140.4 ± 10.3	—	—	—	—
4	Control	130.2	147.1 ± 7.2	5.20	5.88 ± .63	3.29	3.72 ± .32
	Experimental	109.8	160.3 ± 7.9	6.55	9.56 ± .66	2.55	3.72 ± 1.98
6	Control	117.6	141.1 ± 8.4	5.46	6.55 ± .53	2.86	3.43 ± .88
	Experimental	135.8	198.2 ± 14.1	6.00	8.76 ± .58	3.36	4.91 ± .38
8	Control	132.0	188.1 ± 16.3	5.97	8.42 ± .75	3.18	4.48 ± .44
	Experimental	125.6	204.7 ± 8.2	5.57	9.08 ± .63	3.26	5.31 ± .52

*Standard deviation.

TABLE IX

Protein and nucleic acid content of the lung in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	118.2	91.0 ± 5.0*	4.21	3.24 ± .25	6.71	5.16 ± .40
	Experimental	108.2	91.9 ± 24.3	8.58	7.29 ± 1.75	5.01	4.26 ± 1.22
2	Control	118.4	157.4 ± [illegible].8	4.55	6.05 ± .94	7.34	9.76 ± .82
	Experimental	117.2	164.1 ± 27.2	7.43	10.40 ± 1.38	7.51	10.51 ± 1.43
4	Control	126.8	190.1 ± 19.3	5.62	8.43 ± 1.11	6.60	9.90 ± 1.62
	Experimental	143.1	253.2 ± 25.8	4.99	8.83 ± 1.71	7.37	13.04 ± 3.53
6	Control	125.9	215.3 ± 14.7	5.96	10.19 ± 1.33	7.10	12.14 ± 1.88
	Experimental	135.1	240.5 ± 23.2	5.53	9.84 ± .57	6.67	11.87 ± 1.12
8	Control	122.1	216.1 ± 12.6	6.52	11.54 ± 1.64	6.72	11.89 ± 1.93
	Experimental	125.4	242.1 ± 15.9	4.54	8.76 ± 1.50	6.64	12.82 ± 1.00

*Standard deviation.

TABLE X

Protein and nucleic acid content of the spleen in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	147.4	70.8 ± 1.8*	8.86	4.25 ± .38	15.32	7.35 ± .74
	Experimental	157.9	68.2 ± 11.4	25.09	10.84 ± 2.74	10.66	4.60 ± 1.30
2	Control	162.8	112.3 ± 5.7	10.18	7.02 ± 1.31	17.56	12.11 ± 1.73
	Experimental	161.4	123.8 ± 3.8	12.92	9.90 ± .84	18.53	14.21 ± 1.10
4	Control	173.6	126.7 ± 17.1	10.66	7.78 ± 1.44	15.80	11.53 ± 1.28
	Experimental	150.6	116.4 ± 5.5	12.68	9.80 ± .88	18.30	14.14 ± 4.31
6	Control	168.2	153.1 ± 8.8	10.99	10.00 ± .75	17.04	15.50 ± 1.43
	Experimental	182.6	140.9 ± 14.7	11.43	8.82 ± .71	18.39	14.19 ± .99
8	Control	180.2	156.7 ± 7.0	11.65	10.13 ± 1.48	17.55	15.27 ± 1.06
	Experimental	161.2	129.4 ± 9.4	9.89	7.94 ± .54	17.22	13.83 ± .91

*Standard deviation.

TABLE XI

Protein and nucleic acid content of the brain in the young male rat

Week of experiment	Animals	Protein concentration (mg./gm. tissue)	Total protein content (mg.)	RNA concentration (mg./gm. tissue)	Total RNA content (mg.)	DNA concentration (mg./gm. tissue)	Total DNA content (mg.)
0	Control	91.25	153.3 ± 8.6*	2.49	4.18 ± .17	2.00	3.36 ± .23
	Experimental	91.78	142.2 ± 6.3	3.64	5.64 ± .52	1.70	2.64 ± .23
2	Control	99.94	174.9 ± 8.3	2.45	4.29 ± .29	1.70	2.97 ± .61
	Experimental	90.52	165.7 ± 5.9	2.69	4.92 ± .38	1.94	3.55 ± .69
4	Control	99.49	186.0 ± 6.9	2.76	5.16 ± .37	1.69	3.16 ± .56
	Experimental	95.02	188.1 ± 9.0	2.33	4.61 ± .22	2.00	3.96 ± .43
6	Control	94.17	176.1 ± 5.4	2.64	4.94 ± .37	1.80	3.37 ± .62
	Experimental	93.48	183.2 ± 8.2	2.63	5.16 ± .27	1.28	2.51 ± .65
8	Control	91.13	176.8 ± 8.2	2.78	5.39 ± .64	1.82	3.53 ± .50
	Experimental	87.73	180.7 ± 6.0	2.34	4.82 ± .22	1.71	3.52 ± .53

*Standard deviation.

that of a group of rats of the same strain exposed to a pure oxygen atmosphere at 210 mm. Hg total pressure by Pepelko (24).

The growth rate curves for the various organs in both groups of rats were similar and compared favorably with those presented by Widdowson and McCance (34). The actual organ weights were also in agreement with those found in the literature (12, 16, 17, 26, 30). Of the eight organs analyzed, only the spleens of the experimental animals grew at a slower rate than did the spleens of the controls. Although there was only the slightest indication that the adrenals of the experimental rats were producing more corticosterone than were the adrenals of the control rats (table XIV), it may have been sufficient to cause the slight increase noted in the adrenal weight per 100 gm. body weight over that of the control rats (fig. 3).

Values for the DNA, RNA, and protein concentrations in the organs of both the control and experimental rats were well within the limits of the values given in the literature (1, 3, 4, 10, 12, 26, 32, 35). The values listed in tables IV through XI under "total DNA content" provide information as to whether a particular organ has increased in weight by net cell division (hyperplasia). If this value is constant, the total number of nuclei in the organ remains unchanged (12, 35). If net DNA synthesis has stopped but the weight of the organ has continued to increase, then the

TABLE XII

Incorporation of ^{14}C-leucine into rat liver protein

Week of experiment	Controls (c.p.m./mg. protein)	Experimentals (c.p.m./mg. protein)
0	38.6 ± 13.1*	36.7 ± 8.3
2	32.3 ± 10.9	32.6 ± 7.4
4	40.1 ± 10.2	33.6 ± 4.5
6	33.9 ± 7.2	38.5 ± 1.7
8	42.6 ± 8.5	45.9 ± 12.2

*Mean ± S.D.

TABLE XIII

Total serum lactate dehydrogenase (LDH) and LDH isozymes from control and experimental animals

Week of experiment	Animals	Total LDH* (units/ml.)	LDH isozymes (% of total activity)				
			No. 1	No. 2	No. 3	No. 4	No. 5
0	Control	1,856	11.6	20.3	—	24.3	43.8
	Experimental	1,822	8.1	20.5	0.5	27.9	43.0
2	Control	1,543	12.0	16.5	—	23.5	48.0
	Experimental	1,459	5.1	14.2	1.3	23.8	55.6
4	Control	1,888	10.4	17.9	trace	22.3	49.4
	Experimental	1,410	10.8	17.0	1.9	28.5	42.0
6	Control	2,107	13.8	17.7	trace	21.1	47.5
	Experimental	1,560	13.2	16.8	1.0	26.1	43.0
8	Control	2,531	17.1	17.2	trace	23.4	42.3
	Experimental	2,204	15.1	12.6	0.2	21.3	50.9

*One unit of LDH = Δ_{OD} of 0.001/min./ml. of serum at 27° C.

TABLE XIV

Adrenal corticosterone in control and experimental rats

Week of experiment	Animals	μg./adrenal	μg./100 mg. adrenal	μg./100 mg. adrenal/ 100 gm. body wt.
0	Control	0.12 ± .05*	1.05 ± .39	1.21 ± .41
	Experimental	0.66 ± .34	4.98 ± 2.46	4.43 ± 1.97
2	Control	0.42 ± .20	2.65 ± 1.11	1.79 ± .74
	Experimental	—	—	—
4	Control	1.18 ± .29	5.93 ± 2.05	2.49 ± 1.17
	Experimental	1.43 ± .39	6.63 ± 1.06	2.11 ± .36
6	Control	1.20 ± .36	5.55 ± 2.44	1.84 ± .38
	Experimental	1.46 ± .66	6.22 ± 2.42	1.42 ± .40
8	Control	1.02 ± .38	4.84 ± 1.98	1.26 ± .49
	Experimental	—	—	—

*Mean ± S.D.

increase is due to cell enlargement (hypertrophy), and the values listed under "total protein content" will likely show a corresponding increase. The rats used in this study had an average age of 30 days at the beginning of the experimental phase. During the first 3 months of age, the livers of both control and experimental animals continued to grow both by an increase in cell number and in cell size (table IV). These results were in accord with those of other workers (12, 35). The testes of both groups of animals also continued to increase by net cell division, or "true" growth, more so than by cell enlargement during the animals' first 3 months of age. This is evident from the fact that total protein content showed signs of leveling off toward the end of the experiment (table V). In the work of Enesco and Leblond (12), cell division had almost ceased by 2 months of age, and cell size along with the total organ weight had reached a constant value at this time. It should be mentioned that these investigators were using rats of the Sherman strain.

Indications of a possible stimulatory effect of the hyperoxic environment were found in heart muscle in which the number of nuclei continued to multiply to 3 months of age in the experimental animals but not in the controls (table VI). Other investigators have shown that during this time, the total number of nuclei in the heart was increasing very gradually, having almost reached its adult value (12, 35). Similar indications of a stimulatory effect were even more evident when the adrenals were examined (table VII). There was a 350% increase in total DNA content of the adrenals of the experimental animals but only a 20% increase in those of the controls. The results also indicate that cell enlargement by net protein synthesis has ceased in the controls 30 days earlier than in the experimental rats. Values for the adrenals of the experimental rats agreed quite well with those of the Sherman rats (12). Finally, a slight stimulatory effect was seen in the kidneys of the experimental animals, which indicated the total number of nuclei in this organ was increasing at 3 months of age (table VIII). The results from the control rats did not show this upward trend and, hence, were more difficult to interpret. Experiments with Sprague-Dawley rats indicate that net cell division in the kidney has stopped after 45 days (35).

Growth of the lung by a net increase in the number of cells has ceased in both the control and experimental animals by the time they have reached 2 months of age (table IX). Increase in organ size by net protein synthesis has also stopped at approximately the same time. These results do not agree with those of Winick and Noble (35), who showed that "true" growth in the rat lung of Sprague-Dawley animals had ceased at 2 weeks of age.

The only instance of a possible inhibitory effect on the oxygen-exposed animals was seen in the spleen. The organs of the control rats continued to grow by an increase in cell number and in cell size at a time when both of these processes had stopped in the experimental rats. Results of Winick and Noble (35) indicate that net cell division occurred in the spleen for 2 more months.

Results reported by Mandel and Bieth (18) indicate that the cell number in rat brain reaches its final adult level at 16 days after birth. Values on the control rats from the present study substantiate their results, but the same conclusions could not be drawn from the oxygen-exposed animals (table XI). Increase in organ weight by an increase in net protein synthesis was almost nonexistent in both groups of animals.

The RNA concentrations in the organs studied remained essentially constant during the 8 weeks of the experiment. These findings were in agreement with the results of Leslie (17) and Winick and Noble (35). Studying growth in the early postpartum rat, Oliver et al. (23) showed that RNA concentration reached a peak at 15 days after birth and then dropped slightly to its constant adult level. The slight upward trends in the concentration of this nucleic acid from the lung and spleen of the control rats (tables IX and X) remain unexplained.

It was thought that the rate of protein synthesis as measured by uptake of ^{14}C-leucine into the liver would decrease with the age of the rat because a slower rate of increase in whole body weight was observed after the animals had reached 2 months of age (table II). This was not true, especially in the experimental animals (table XII). Although the body weight had increased at a slower rate during the latter 4 weeks of the experiment, two other factors which might contribute to this increased rate of protein synthesis should be considered. Because of the turnover rate of the many different proteins in the body, much of the rat's protein begins to require replacement as the rat grows older. Also, because the liver is the major site of plasma protein synthesis (23), a greater demand for new protein will be placed on the liver as the volume of plasma increases. Thus, as the rat grows older, it must synthesize new protein not only for structural requirements, but also for replacement in the tissues and in the increasing volume of plasma.

The LDH isozymes were studied in rat tissues up to 45 days after birth by Blatt et al. (6). These investigators showed that the predominant subtype at birth was the muscle form (LDH-5), suggesting anaerobic glycolysis, and that development of the animal was characterized by an increase in the ratio of heart subtype (LDH-1) to muscle (LDH-5) in all tissues studied. In the present investigation, examination of the serums of the growing animals for alterations in their isozyme patterns showed a slight upward trend for LDH-1 as the animals developed. There was a slight but significant amount of hemolysis in the serum samples which tended to mask any additional changes. As for the elevation in total LDH activity of the controls with time, it is known that the erythrocyte of the rat, containing 100 times as much LDH activity as the serum (7), is very fragile and undergoes hemolysis quite readily (2). A young rat, 30 days of age, has a younger, less fragile population of erythrocytes than does a 90-day-old rat; hence, the erythrocytes of the older rats would have a greater tendency to hemolyze and effectively elevate the total LDH activity in the serum.

The relative humidity in the chamber housing the oxygen-exposed animals had an average value of 45.7%, whereas the control chamber

averaged 72.7%. Examination of the protein concentrations of the various organs (tables IV through XI) indicated that the experimental animals had not become dehydrated during the 8 weeks of exposure to this lower humidity. Had the kidneys not been able to compensate by excreting less water in the urine, the protein concentration of the various organs would have increased.

The question as to whether the parameters chosen in this study adequately describe growth and the many ramifications of such a process are open to discussion. By examining the results obtained with both groups of animals during the 8 weeks of the experiment, it is concluded that the oxygen-exposed animals were not adversely affected by the hyperoxic environment.

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13 ABSTRACT

Young male rats were maintained continuously in a hyperoxic environment for periods up to 8 weeks. During this time, the animals were subjected to an oxygen partial pressure of 369 mm. Hg (97.1%) at a simulated altitude of 380 mm. Hg (18,000 ft.). The parameters chosen to delineate growth and development in the rat were: total body weight gain; weight gain of the liver, kidney, testis, spleen, lung, adrenal, heart, and brain; deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein content of these organs; incorporation of ^{14}C-leucine into liver protein; and serum lactate dehydrogenase (LDH) isozymes. In spite of the changes reported in the text, the animals grew well in this atmosphere; however, it should be noted that several fundamental changes were observed in the growth and development on the cellular level.

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