

SOME METABOLIC EFFECTS OF HIGH OXYGEN CONCENTRATIONS
IN RELATION TO RETROLENTAL FIBROPLASIA

by

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II. INTRODUCTION

Retrolental fibroplasia is a blinding disease of prematurely-born human infants of low birth weight, involving the retina of the eye. The disease, if it can be called such, involves the abnormal vascularization of the retina, with subsequent retinal detachment and thus, blindness. After detachment, the retina becomes a fibrous mass behind the lens of the eye - thus the name, retrolental fibroplasia.

This malady was unknown to ophthalmologists and pathologists until 1941. In February of that year, Dr. Theodore J. Terry, an ophthalmologist and pathologist of the Harvard Medical School, found and reported the first known case of retrolental fibroplasia in medical history. Soon after Dr. Terry's discovery, numerous cases came to the attention of the ophthalmologists in the Boston area and in adjacent areas. Dr. Terry alone had 117 cases of retrolental fibroplasia in 1941.

The disease seemed to become relatively widespread within a short period of time. A very peculiar aspect of the problem was the fact that infants born in the larger and better-equipped medical centers of the country were the ones most frequently afflicted. Not only were the prematurely-born infants given the best of medical care, but their mothers had received excellent prenatal care.

Investigations to determine the cause of retrolental fibroplasia were at first directed toward possible nutritional deficiencies in the mother which could cause blindness in her infant. None of the investigators were able to demonstrate that maternal nutritional deficiencies were a causitive factor.

In 1947 and 1948 several quiet investigations were undertaken to determine whether or not high oxygen concentrations administered to prematurely-born infants could be a factor in causing this blinding disease. The results of these investigations seemed to show that it was definitely a factor, but few ophthalmologists and other investigators were ready to accept such findings. It was becoming evident that more controlled studies were needed and the number of infants used should be greatly increased.

In 1952, Dr. Everett Kinsey was instrumental in forming the Co-Operative Study of Environmental Oxygen and Retrolental Fibroplasia. This study was begun in 1953, with 18 hospitals participating in the study, and almost 800 infants being used in the investigation. The hospitals were divided into two groups: one group was to administer the routine high oxygen concentration to the premature infants of low birth weight, while the second group was to administer oxygen only when absolutely

necessary to save the life of the infant, and then in concentrations not to exceed 40%.

At the end of this study, it was determined that approximately 25% of the infants receiving high oxygen concentrations had suffered blindness as a result of retrolental fibroplasia. Only 7% of the infants on restricted oxygen therapy developed the disease.

The disease has been practically eliminated in those hospitals which greatly curb the use of oxygen for prematurely-born infants of low birth weight. Rare cases have been reported in infants who received no oxygen.

Even though it has been shown that oxygen is the major cause of retrolental fibroplasia, the mechanism of its action in this malady is not understood. Furthermore, many of the investigations concerning it have been carried out from the standpoint of maternal factors, other than factors which may exist only in the newborn infant.

It is the object of this investigation to expose newborn experimental animals to high oxygen concentrations and observe the metabolic effects. It is hoped that the findings will give some evidence as to the mode of action of high oxygen concentrations on certain tissues and metabolites, and the way in which this action may be a cause of retrolental fibroplasia.

III. REVIEW OF THE LITERATURE

Etiology and Pathogenesis of Retrolental Fibroplasia

Terry (1) in 1942 wrote the first descriptions of retrolental fibroplasia as an entity. He also gave the disease its name and was the first to point out the etiologic factor of prematurity. However, it was Terry's belief, after his early observations of infants with the disease, that the fibrous mass behind the lens of the eye was the remains of the embryonic hyaloid artery which had become thickened and fibrous (2). He was of the opinion that this disease entity consisted of primary and secondary changes related to 1) the persistence and overgrowth of the hyaloid artery system, 2) growth of embryonic connective tissue behind the lens of the eye, 3) persistence of overgrowth of fibrilla structures of vitreous (3).

As late as 1946, it was considered that none of the various classes of etiologies could be excluded. These included heredity, causes operating before birth, those arising from premature separation from maternal hormones, the precocious functioning of such systems as the respiratory, digestive and cardio-vascular, the "heat regulating center," and those arising through the precocious exposure to light (4), (5).

Owens and Owens (6) first described the clinical pathogenesis of the disease. They reported that in the first stage of the disease the blood vessels of the retina swell to three or four times normal thickness and twist into tortuous hairpin curves. This is followed by an abnormal proliferation of the capillaries, a swelling and detachment of the retina, and finally a cicatricial stage characterized by the detached retina forming into a disorganized mass behind the lens. This process may be very violent and rapid, or it may be slow and smoldering and may stop at any stage.

Patz (7), Friedenwald (8), Krause (9) and Heath (10) have all added to these observations as well as confirmed them.

Huggert (11) contributed significantly to the understanding and knowledge of the maturation of the vascular system of the human eye. He found that infants born weighing less than 2,000 gm. at birth had embryonal vascular structures and vitreous clouding which disappeared in two to three weeks. It is very significant to note that he found infants born weighing less than 1,601 gm. not only had these embryonal vessels, but frequently had thread-like retinal vessels in the first weeks after birth. These retinal vessels grew in size to normal within five to seven weeks in the smaller, and within two to three

weeks in the larger infants. Half of these infants with early vitreous clouding and thread-like retinal vessels later had dilated vessels, usually followed by peripheral retinal clouding, edema, or in some instances, detachment and neoplasia. The other half of this group of premature infants showed normal ocular development. Two of the smaller infants developed cicatricial retrolental fibroplasia, but the remainder of the 130 infants seemed to have normal eyes. Huggert has shown, then, that growth and development of the retinal blood vessels progresses according to an orderly preset pattern, more closely related to weight than to age after birth (11).

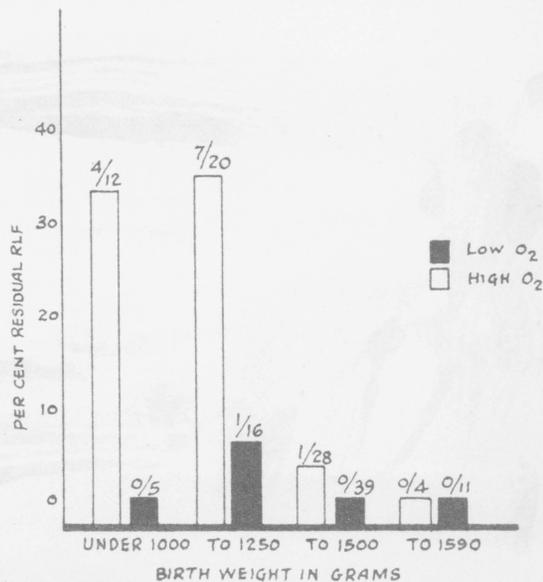
It becomes apparent from this investigation that retrolental fibroplasia arises during a definite stage of ocular development. The typical weight of a human infant at the onset of retrolental fibroplasia is 1,800 to 2,200 gm. (12).

Thus, investigations were directed to finding factors which disturb this orderly process of development of the retinal vascular system and lead to retrolental fibroplasia through abnormal capillary proliferation.

Kinsey and Zacharias (13) were one of the first groups to call attention to the frequency of use and duration of exposure to oxygen as a possible causitive factor.

In 1948, Patz and Hoeck (14) undertook a quiet investigation, based on an isolated observation, to pursue the possibility that oxygen might be related to the disease. These workers obtained a striking correlation between the number of days of oxygen therapy and the incidence of retrolental fibroplasia. However, prolonged oxygen therapy could not be accepted as a causal factor from their data since there was no randomization of cases and no controls were used (14).

These two workers initiated another study in 1951, instituting rigidly controlled randomization (7). Premature infants were placed in either a high oxygen group or curtailed oxygen group. Those infants in high oxygen received 60 to 70 per cent oxygen concentrations for 28 days or longer; those in the restricted oxygen received concentrations usually under 40 per cent and only for specific clinical indications. Their findings are illustrated in the chart shown below:



Patz, et al (7), (14) were able to demonstrate the characteristic microscopic changes of human retrolental fibroplasia in newborn rats, mice, kittens and puppies by exposing them to 60-80 per cent oxygen concentrations for four days or longer.

The characteristic early changes of retrolental fibroplasia occur in the vessels of the nerve fiber layer, thus the degree and extension of vascularization of this layer is of central interest. In mice, this vascularization takes part entirely after birth, making the animals appropriate for experimental use (17). In the rat, the vascularization and development of the retina at four days corresponds to that of a seven month human fetus (14).

While Patz and Hoeck (7) were undertaking their controlled studies, confirmation of the observation that the incidence of retrolental fibroplasia increased with duration of exposure to oxygen was reported from Australia by Campbell (18) and from England by Crosse (19).

In 1952 the National Co-Operative Study on Retrolental Fibroplasia was initiated in an effort to evaluate on a national scale any lead for the solution of the problem of retrolental fibroplasia (20). Eighteen hospitals joined in this study and over 700 prematurely-born infants

were entered into the investigation. The infants were divided into two groups; the first group to receive oxygen in concentrations of over 50 per cent for 28 days, while the second group was to receive either no oxygen or limited amounts prescribed only on the basis of urgent clinical need. The study involved only infants who survived 48 hours and those weighing less than 1,500 grams or less at birth. At the end of the first three months of the investigation, it was found that 25% of the infants given routine high oxygen concentrations had developed retrolental fibroplasia, whereas only 6% of those on curtailed oxygen therapy developed the disease. Percentage of mortality was not significantly higher in the curtailed group. However, in summation, Kinsey did not imply that oxygen itself was the sole cause of the disease, but rather that it is associated with the true cause (20).

Active and cicatricial grades of retrolental fibroplasia have been classified by Reese (21), King (22) and Owens (23). It seems relevant to list these stages in view of the material that follows. The stages now recognized may be summarized as follows:

Stage I The vascular stage characterized
by tortuosity and dilation of
retinal vessels. Vessels reach

a size 3 or 4 times normal diameter. At the end of the vessels fine, twig-like, delicate vessels can be seen.

- Stage II The vitreous becomes hazy and neovascularization becomes more profuse. There is occurrence of retinal hemorrhage.
- Stage III Fine strands of newly formed vessels with their supporting tissue can be seen extending like veils into the vitreous from localized areas of retinal elevation. Localized detachment of the retina occurs.
- Stage IV With further progression, more of the retina becomes involved. At this stage at least half the retina is involved.
- Stage V This stage involves the entire retina and often a massive hemorrhage occurs filling the entire vitreous. Varicose capillaries are found in whorls and the vascular proliferation bursts out of the retina.

Vascular proliferation is normally most active in the sixth and seventh months of fetal life, and those infants who at premature birth still have their retinal vascularization to be completed are those susceptible to the disease (24).

Patz (14) has pointed out that the hemoglobin saturation of oxygen of the fetus in utero is approximately 50%. At birth, at ordinary room atmosphere, this saturation suddenly reaches 90%. He believes that in cases of retrolental fibroplasia (RLF) where the infant has been exposed to no oxygen or in cases where there is retrolental fibroplasia in a full-term infant, this sudden increase in the hemoglobin saturation of oxygen could be a causitive factor.

Oxygen administration apparently elevates the choroidal oxygen tension, resulting in increased diffusion across the retina to eliminate the normal anoxic growth stimulus. The retinal vessel growth is then suppressed and the vessels become attenuated or obliterated. When growth is ultimately activated, the normal channels of vascularization probably no longer exist and the vessels erupt into the vitreous (14). This hypothesis evolved after experiments were conducted to substantiate the toxic effect of oxygen in RLF as opposed to Szewczyk's belief that retinal changes in the

disease are the response of immature neural tissue to slight anoxia. Szewczyk observed that when infants acclimated to 50% oxygen over a period of several days were abruptly withdrawn to ordinary levels (22%) in room air, the retinal vessels dilated and hemorrhage and edema of the retina appeared within a few days. When the infants were returned to high oxygen concentrations, prompt regression of the condition was noted. These changes were interpreted as resulting from inadequate oxygenation of the blood induced by exposure of the premature infant to an environment of higher oxygen concentration and withdrawing him so rapidly that the physiological changes of acclimation could not take place before rapidly differentiating areas such as the retina of the eye had been harmed. Jefferson (26) confirmed Szewczyk's observations. However, Patz (7) et al were able to demonstrate these changes by mere exposure of infants to high oxygen concentrations and with no subsequent sudden withdrawal from the conditions.

It is not yet settled whether the etiologic mechanism should be considered as a relatively simple toxic effect of hyperoxia or to a more complex one involving body temperature fluctuations, electrolyte shifts, hemoglobin concentration, sudden changing of atmosphere and, ultimately, hypoxia (27). There is

strong evidence, however, of a much more complex mechanism and of a chain of circumstances leading to retinal hypoxia (28), (25), (29), (26), (30), (31), (32), (33).

Tissue anoxia (hypoxia) may occur in an indirect manner. Himwich (32) and others (34), (35), (36), (37) have pointed out that the carrying power of blood for carbon dioxide is diminished during exhalation of oxygen at excessive pressures; this in turn brings on an acidosis that may contribute to tissue anoxia. Himwich has an exceedingly interesting hypothesis concerning a mechanism by which excessive oxygen might bring about histoxic anoxia by changing the sulfhydryl-containing enzyme involved in the oxidation of pyruvic acid (pyruvic oxidase). He believes the hydrogen may be oxidized away from the SH groups, incapacitating the enzyme, and thus preventing further glucose oxidation. With glucose oxidation ceasing at the pyruvic acid level, he believes a condition of cellular or histoxic anoxia may be produced.

Ingalls (27), (28) has pointed out that the importance of defining whether the ocular lesion is due to tissue hyperoxia or tissue anoxia is two-fold: because a primary form of anoxia may be overlooked and because an improved understanding of RLF will possibly contribute to the

understanding of the genesis of congenital anomalies.

The mammalian newborn shows an extraordinary tolerance to anoxia as compared to the adult (38). The immature brain has the capacity to survive for relatively long periods without oxygen by obtaining energy anaerobically through glycolysis. Himwich (32) has shown that both anaerobic and aerobic types of metabolism increase as growth proceeds but that oxygen consumption, an indication of aerobic metabolism, soon increases above the level of anaerobic glycolysis. This shift from the anaerobic to the aerobic source of cerebral energy is found to be the result of enzymatic changes, owing to the appearance of new enzyme systems and also to increased concentrations of enzymes already present.

Ashton and Cook (39) have greatly contributed to our knowledge of the developing retina and the developing retinal vessels. Undoubtedly their work will be invaluable in the attempt to correlate the incidence of RLF in the undeveloped eye with etiologic factors. These workers were able to cause intravitreal proliferation in kittens by first detaching the retinas. There was great similarity between these proliferations and those seen in Stage I retrolental fibroplasia. Thus it appears that retinal detachment may play a role in cases of RLF, and especially those in which no supplemental oxygen has been given.

Thus far in this review no consideration has been given to investigations involving possible nutritional factors indicated in retrolental fibroplasia, although much of the early work concerned with the disease centered about possible maternal nutritional deficiencies which might induce such an anomaly in the newborn.

This part of the discussion has been reserved for the latter part of the review so the reader may better follow the rationale behind the present investigation. It seems pertinent to have given the pathogenesis of the disease in the preceding pages.

Warkany (40), in 1942, reported congenital malformations induced in rats by maternal nutritional deficiencies. In a later, more extensive investigation in collaboration with Schraffenberger (41), Warkany undertook a study of the possible role of maternal vitamin A deficiency in RLF. Female rats were raised on a diet of known small carotene content, bred while on a highly purified diet free of carotene and vitamin A, and were mated with normal males. The young born to those females capable of reproducing living offspring were born blind and with deformed eyes. In addition to these abnormalities of the eyes there were defects of skeletal and soft tissues. Histologic sections of the eyes were studied and a fibrous retrolenticular membrane was found in every specimen.

However, Warkany and Schraffenberger (41) concluded, "Ocular defects occur in the young rat only when the maternal vitamin A deficiency is extremely severe to the extent that the physiological processes associated with reproduction in the human being parallel those in the rat, it may be inferred that vitamin A deficiency is not a probable cause of retrolental fibroplasia."

It seems exceedingly peculiar that Warkany and Schraffenberger should omit the probability that vitamin A destruction might occur in the infant even though maternal and infant levels and stores are optimal at birth.

Rollet and Edel⁽⁴²⁾ have reported vascularization of the cornea as one of the first signs of avitaminosis A.

Experimental evidence has been reported indicating that the administration of alpha-tocopherol reduced the incidence of retrolental fibroplasia, but subsequent work has not shown vitamin E to significantly effect the onset of the disease (6).

Kinsey (20) recently reported, ". retrospective studies in relation to infants have shown a positive association between the incidence of retrolental fibroplasia and the administration of any of the following: water-miscible vitamins, iron, oxygen, and blood transfusions; other factors which at

one time or another have been thought to be associated with retrolental fibroplasia include virus infection, lack of hormones in the infant, premature exposure of the infant's eyes to light, and lack of vitamin A in the mother. All of the factors mentioned above, except the administration of oxygen, have been largely eliminated as likely etiological agents either through controlled clinical tests or further retrospective examination of the records."

Hepner (12) reports two studies in which infants weighing less than 1500 gm., given 20 mEq. or less of sodium per liter in cow's milk formulae, show a very low incidence of RLF. Where similar formulae containing 56 mEq. of sodium per liter were fed infants weighing less than 1500 gm., over 43% became blind from RLF.

It seems evident from reviewing the mass of literature concerning studies on RLF that comparatively little work has been done concerning the possible effects of oxygen on the chemistry of the tissues either directly or indirectly involved in the disease. One aspect that appears to warrant further study is that of chemically examining the tissues of newborn mammals after their exposure to high oxygen concentrations in an effort to find what metabolites may be depleted or rendered unavailable and their possible action in the function of normal tissue.

IV. EXPERIMENTAL

Experiment I

A. Methods and Materials:

White female rats of the Holtzman strain were maintained from weaning on a stock ration, with all dietary essentials being supplied at an optimal level. The composition of the stock diet was casein 12%, wheat 56%, meat meal 10%, skim milk 8%, alfalfa 2%, salt 0.5%, Quadrex 0.8%, molasses 5%, and Crisco 5%. The Quadrex contained vitamin A, 5000 USP units per gram; vitamin D₂, 625 USP units per gram; not less than 5% crude protein; not less than 8% crude fat, in addition to 8% crude fiber and 8% ash. These females were bred with select males of the same strain and were fed the stock ration during the entire gestation period. The young were weighed during their first day of life, the 4th and 7th days, and at weekly intervals thereafter.

At four days the young and the mother rat were placed in a gas-tight chamber and maintained in an atmosphere of 70 or 100% oxygen. Water and stock ration were placed in the chamber. When the experimental animals were not maintained in an atmosphere of 100% oxygen, they received a mixture consisting of 70% oxygen, 20% nitrogen

and 5% carbon dioxide. The pressure within the chamber was kept constant and there was a continuous flow of gas through the chamber. The temperature of the room in which the chamber was placed was maintained between 75°F and 80°F. No means was available to check humidity within the chamber.

The chamber was constructed of $\frac{1}{2}$ " plywood and treated with acid-resistant paint. A window was placed on one side of the chamber in order to observe the feeding habits of the mother animal and her care of the young.

The young rats were kept in the chamber for intervals of 7, 10, or 14 days. Mother animals were removed from the chamber every 48 hours and exchanged with the mother rat of the control group. In this manner it was possible to check the lactation of the mother animal which had been exposed to the oxygen and to observe the weight gain of the young rats nursing such a mother. Thus, both control and experimental young were fed by mother animals which had been exposed to high O₂ concentrations. Only rarely did cannibalism result from this interchanging.

It was necessary to replace the exposed mother every 48 hours in order to prevent pulmonary edema, pneumonia or other disorders which other investigators have previously reported in adult animals (43). The young suffer no such consequences from the exposure. The

experimental young were exposed to room atmosphere only during the period when the mothers were being exchanged. This averaged about 10-15 minutes every 48 hours.

Control animals used were those born of mothers of the same strain as the experimental group, maintained on an identical diet, and in most instances were those born on the same day as the animals used in the experimental group.

Experimental animals were sacrificed by decapitation not more than one hour after being removed from the chamber. There was little opportunity for an "acclimation" period from time of removal to time of sacrificing. Brain and liver tissues were rapidly taken from the animals, blotted with filter paper and placed in clean, covered watch glasses away from light. When it was impossible to immediately analyze the tissues, they were kept frozen at -10° to -15° C.

A short study was done to determine the liver storage level of ascorbic acid of white rats at birth and the rate of synthesis in the young, growing animal. Results of the study are given in Table I.

The method used for the determination of vitamin A in both brain and liver tissue is the modified and simplified Carr-Price reaction of Ames, Risley and

Harris (44). The modifications necessary in employing this method involved the use of proportionally smaller quantities of ethyl ether and sodium sulfate for smaller quantities of tissue. The liver size in young rats of 11-18 days old prohibited using tissue quantities of 3-5 grams as was used in the original method. All other reagents and instruments used in the analyses were identical with those designated in the method of Anes et al (44).

Ascorbic acid determination of liver and brain tissue was by a modification of the Schaffert and Kingsley (45) method of ascorbic acid determination of whole blood. No satisfactory method for ascorbic acid determination of tissue, as such, could be found in the literature. It was impossible to satisfactorily obtain enough blood from the young rats for blood analysis. Modifications of the Schaffert-Kingsley method included merely the use of 10 cc of 6% trichloroacetic acid per gram of tissue instead of 15 cc of 6% trichloroacetic acid per 5 ml. blood, a reduction in the quantity of Norit used, and in incubation period of 10 minutes at 100°C. Samples were read in an Evelyn colorimeter using a 520 mμ filter.

B. Results:

The liver ascorbic acid level was affected only in the group which received 100% oxygen for a period of ten days. It can be noted from Table II that this same oxygen concentration administered for a period of seven days had no effect on the tissue level. Animals which were exposed to a 70% O₂ concentration for periods of ten or fourteen days showed no loss of liver ascorbic acid.

Brain ascorbic acid was appreciably reduced by exposure of the animals to both 100% and 70% oxygen concentrations. It is significant to note from Table III that at fourteen days in 70% O₂ the animals showed no loss, whereas at ten days there was a loss of 93.4 ug ascorbic per gram of tissue. It appears then, that there is a critical period in which the brain tissue is markedly affected. The results of this experiment indicate that this critical period terminates sometime after the fourteenth day of life (four days plus ten days' exposure). Table III shows that the greatest degree of depletion in brain tissue occurs before the 14th day of age in the rat.

The brain ascorbic acid levels obtained on analysis of tissue from the control animals compare very favorably with those obtained by other workers (46), (47).

In studies thus far undertaken, no loss of vitamin A has been detected in liver tissue of animals identically exposed. However, these studies are yet in the preliminary stage and the results obtained thus far should not be considered conclusive in any respect.

In analyzing tissue of several of the control groups such small quantities of tissue were available that often the vitamin A content was below the level which could be accurately determined by the method used. Thus, the results of the vitamin A study cannot be emphasized, although it seems somewhat indicative that the tissue levels of the experimental animals remained relatively constant regardless of the exposure period. The results of the vitamin A study are summarized in Table VI.

It is unfortunate that the animals used were necessarily so young and so small. This made it difficult to obtain large enough quantities of tissue from each animal for accurate analysis of both ascorbic acid and vitamin A.

Experiment II

A. Methods and Materials:

Since Experiment I indicated a rather appreciable loss of total ascorbic acid in brain tissue after exposure of the animals to a 100% oxygen concentration, it seemed pertinent to determine whether this loss was from ascorbic acid destruction or from the possible blocking of some synthetic pathway. The simplest procedure for such a determination seemed to be the use of an animal which cannot synthesize ascorbic acid. It was thus reasoned that if such an experimental animal showed a tissue loss of ascorbic acid after exposure to high O_2 concentrations, the loss would necessarily indicate destruction. Of the two animals that require a dietary source of ascorbic acid (man, other primates and the guinea pig), the guinea pig was chosen as the experimental animal for Experiment II.

The guinea pigs were of commercial stock and obtained from the Manor Farms of Staatsburg, New York. The average weights of the groups used in each experiment appear in the tables on pages 31, 32, 33, 34, 35, and 36.

The experiment was conducted by dividing the guinea pigs into groups of the following order:

Group I received 70% oxygen for a period of seven days and no dietary supplement of ascorbic acid was given either the control or experimental animals.

Group II received 70% oxygen for a period of 10 days but head lettuce was added to the diet of both control and experimental animals. Each animal was given 50-55 grams head lettuce every other day.

Group III received 100% oxygen for a period of seven days, with head lettuce again constituting the dietary source of ascorbic acid.

All of the lettuce given the animals was consumed and from this supplement they received a minimum of 2 mg. ascorbic acid per day. This estimate is based on data from Peterson and Strong (1) which give the ascorbic acid content of head lettuce as 8 mg. per 100 grams of the edible portion. The animals were given a diet of Purina pellets and water, with or without lettuce (as the experiment indicated). They were pair-weighed so that control and experimental groups would be comparable.

Experimental procedure concerning the administration of oxygen was identical with that used with the white rats. However, it must be pointed out that the guinea pigs were of weaning age and therefore comparatively older than the young rats used. The animals were placed in the same

chamber as described in Experiment I, and the gas mixtures were also identical with those used in that experiment. The animals were removed from the chamber for a few minutes every 48 hours for cleaning and feeding purposes. The control groups were also treated in this manner - food and water being replenished every 48 hours. At the end of the experimental period, animals were removed from the chambers, weighed and immediately sacrificed by anesthetizing with ether. Tissues were removed, blotted and stored by freezing when immediate analysis could not be made. Control animals were sacrificed at the same time and by the same method.

Ascorbic acid analysis of the tissues was by the identical method used in Experiment I with the same modifications employed. Whole blood analyses were by the Schaffert and Kingsley method (45), unmodified.

During the course of the experiment, the experimental animals of Group II developed what appeared to be an abnormal eye condition. These eyes were examined by Dr. W. B. Gross of the poultry pathology department, Virginia Polytechnic Institute, during the experiment, and at the time the animals were sacrificed the eyes were removed, placed in Bouin's solution and later prepared for histologic examination. Eyes from the control animals were also removed and prepared. The results of the pathologic

and histologic studies appear in the following section.

B. Results:

Ascorbic acid in liver tissue of these young guinea pigs was depleted 34% - 50% by exposure to high oxygen concentrations. It is difficult here to determine whether 70% or 100% oxygen caused the greatest depletion since the various groups tested were of somewhat different ages and weights, and no attempt was made to ascertain the loss on a per-cent-body-weight basis. However, it should be pointed out that in all cases the experimental group and control group in any particular experiment were of comparable weights. The important factor is, of course, the difference in level of experimental and control group in any single analysis.

Although the gross loss of ascorbic acid in the white rats may seem appreciable, it can be noted from Table IV that the loss in the guinea pigs represents a far greater loss, on a percentage basis.

Brain ascorbic acid levels of the guinea pigs were not as severely depleted as in the liver tissue, although the depletion of brain ascorbic acid in experimental animals ranged from 14% to 21% (Table V). This is somewhat surprising as the brain tissue in the white rats showed a more consistent depletion on exposure than did the liver tissue.

Pathologic and histologic studies of the eyes of these experimental animals showed degenerative changes in the nerve fiber layer of the retina and there appeared to be a reduction of the number of cells in the layer of the ganglion cells. There was also evidence of degenerative changes in the layer of the rods and cones.

Ophthalmological examination of the animals' eyes just before they were sacrificed showed a type of coronary cataract involving the surface layers of the lens.

TABLE I

Liver Ascorbic Acid Levels of Normal White Rats
at Various Ages

Age in Days	ug Ascorbic Acid Per Gram Fresh Wt. Tissue	
0- 1	180.8	(4)
2	191.3	(5)
4	216.0	(4)
9	291.6	(5)
14	325.0	(4)
18	373.0	(4)

Figures in parentheses denote number animals used in study.

TABLE II

Liver Ascorbic Acid Levels of Young White Rats
Exposed to High Oxygen Concentrations

% O ₂	No. Days in Exposure	Age in Days	ug Ascorbic Acid Per Gram Fresh Wt. Tissue Experimental Group	ug Ascorbic Acid Per Gram Fresh Wt. Tissue Control Group (No. O ₂)	Tissue Loss Per Gram
100	7	11	285.0 (5)	283.6 (5)	0.0
100	10	14	150.4 (4)	325.0 (4)	174.6
70	10	14	312.5 (6)	300.0 (4)	0.0
70	14	18	382.0 (5)	373.0 (4)	0.0

Experimental animals 4 days old on exposure.

Figures in parentheses denote number animals used.

TABLE III

Brain Ascorbic Acid Levels of Young White Rats
Exposed to High Oxygen Concentrations

% O ₂	No. Days in Exposure	Age in Days	ug Ascorbic Acid		Tissue Loss Per Gram
			Per Gram Fresh Wt. Tissue Experimental Group	Per Gram Fresh Wt. Tissue Control Group (No. O ₂)	
100	7	11	554.6 (5)	612.5 (5)	57.9 mg
100	10	14			
70	10	14	481.6 (6)	575.0 (4)	93.4 mg
70	14	18	434.0 (6)	426.6 (4)	0.0

Experimental animals 4 days old on exposure.

Figures in parentheses denote number animals used.

TABLE IV

Liver Ascorbic Acid Levels of Young Guinea Pigs
Exposed to High Oxygen Concentrations

% O ₂	No. Days Exposure	ug Ascorbic Acid Per Gram Fresh Wt. Tissue Experimental Group		ug Ascorbic Acid Per Gram Fresh Wt. Tissue Control Group (No. O ₂)		Tissue Loss Per Gram
*100	7	27.5	(2)	41.9	(2)	14.4 mg
**70	7	37.5	(3)	66.8	(3)	28.3 mg
**70	7	35.0	(3)	71.2	(3)	36.2 mg
*70	10	37.5	(3)	57.0	(3)	19.5 mg
						34%-50% loss

Figures in parentheses denote number animals used.

* Ascorbic acid included in diet.

** No ascorbic acid included in diet.

TABLE V

Brain Ascorbic Acid Levels of Young Guinea Pigs
Exposed to High Oxygen Concentrations

% O ₂	No. Days Exposure	ug Ascorbic Acid Per Gram Fresh Wt. Tissue Experimental Group	ug Ascorbic Acid Per Gram Fresh Wt. Tissue Control Group (No. O ₂)	Tissue Loss Per Gram
*100	7	95.0 (2)	120.6 (2)	25.6 mg
**70	7	112.5 (3)	131.8 (3)	19.3 mg
**70	10	86.0 (3)	102.5 (3)	16.5 mg

*Ascorbic acid included in diet.

**No ascorbic acid included in diet.

Figures in parentheses denote number animals used.

TABLE VI

Vitamin A Levels of Young White Rats
Exposed to High Oxygen Concentrations

% O ₂	No. Days Exposure	Age in Days	ug Vitamin A per gram Fresh Wt. Tissue	
			Experimental Group	Control Group
100	7	11	176.6 (8)	*130.0 (7)
100	10	14	174.3 (4)	*139.0 (4)
70	10	14	121.2 (4)	173.3 (3)

Experimental animals four days old on exposure.

Figures in parentheses denote number animals used.

*quantity of tissue used in analyses did not contain enough
vitamin A for accurate determination.

VI. DISCUSSION AND CONCLUSIONS

It is somewhat disconcerting that newborn white rats showed a loss of brain ascorbic acid at both concentrations until their fourteenth day of life. There was no loss of liver ascorbic acid in the groups receiving 70% oxygen, but an appreciable loss occurred in the group receiving 100% oxygen. If we can interpret the results of Experiment II, in which the guinea pigs showed a consistent loss, as pointing to the destruction of ascorbic acid and not the blocking of synthesis, then it would appear that there is some factor present in liver tissue of the rat which prevents this destruction by oxygen until the concentration nears 100%. Brain tissue of the rat must lack this factor.

It has been suggested by several authors (43), (25) that exposure of animals to high oxygen concentrations may lead to the inactivation of sulfhydryl compounds, particularly sulfhydryl enzymes. Glutathione, the widespread tissue sulfhydryl, is thought to be a "protector" of ascorbic acid because of the ease with which it is oxidized. Hassan and Lehninger (48) report that reduced glutathione reduces the rate of destruction of ascorbate but does not increase the rate of formation from glucuronate in rat liver. It is possible then, that liver

tissue may either contain an additional factor which protects ascorbic acid, as previously mentioned, or there may be present appreciable quantities of a "protector," which is absent in brain tissue. It may be more probable, however, that the rate of synthesis in the liver is much greater than that in the brain. It should be pointed out that the results of these experiments show brain tissue ascorbate to be approximately 0.1 to 0.4 mg. per gram higher than the liver ascorbate of the rat.

From the results of this experiment (Table III), there is apparently some factor in brain tissue of the rat which emerges after the fourteenth day of life and prevents the destruction of ascorbic acid by exposure to excessive oxygen. Table III also shows a reduced level of brain ascorbate after this period. In view of the problem of retrolental fibroplasia, and the fact that the disease grossly affects the undeveloped eye, this early high level of brain ascorbate which diminishes may indicate its necessity for the normal development of the retina and other structures which are not complete at the birth of the young. It seems significant that the brain level diminishes from time of birth and reaches a plateau after the 14th day in the growing young.

The higher percent loss of tissue ascorbate in the

guinea pig as contrasted to the rat is, of course, expected as the rat could at least partially overcome the loss by resynthesis. The most outstanding result as far as comparing the two animals is that which shows the guinea pig liver tissue to be depleted by both concentrations of O_2 with a very high loss, whereas the liver tissue of the rat was the least affected of all tissues analyzed. Again, this seems probably due to a high rate of synthesis in rat liver.

The histologic observations from sections of the experimental guinea pig eyes demonstrate again (14) the ease with which these abnormalities can be induced in mammals by exposure to high O_2 concentrations. More important to this experiment, though, is the possible correlation between the appearance of the abnormalities and the depletion of tissue ascorbic acid. It is unfortunate that lack of facilities and personnel at the time of the experiment prevented the histologic study of the rat eyes also.

VII. SUMMARY

Newborn rats and young guinea pigs have been exposed to high oxygen concentrations with subsequent analyses of ascorbic acid in liver and brain tissue.

Newborn rats show a depletion of liver ascorbic acid only after exposure to 100% oxygen. Extending the exposure period in 70% oxygen did not result in any depletion.

Brain ascorbic acid levels in newborn rats were depleted by exposure to both 100% and 70% oxygen until the 14th day of life. After that age, exposure failed to cause depletion.

Guinea pigs identically exposed showed ascorbic acid depletion in both brain and liver tissue with the more marked depletion occurring in liver tissue.

Abnormalities in the eyes of the experimental guinea pigs were demonstrated.

Results of the tissue Vitamin A analyses are incomplete and inconclusive at this time.

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