STUDIES ON THE OXYGEN CONSUMPTION
OF NON-NUCLEATED ERYTHROCYTES

DISSertation

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INTRODUCTION

In general, the long-range plan for these and other studies in this laboratory is to correlate with the metabolism of the respective cells and tissue, certain general physiological properties, like membrane permeability and possibly membrane potentials, which are known to be altered in the wake of adrenocortical insufficiency. In particular, these investigations, the results of which are to be presented, are limited to the metabolic aspect of the foregoing problem. The studies treat of the role of a physical agent (x-irradiation) known to effect the permeability and metabolism of cells and tissues (Duggar, 1936) and of the possible role played by the adrenal cortex in alleviating the induced changes. Though the x-irradiation effects on permeability and cellular metabolism are clear enough to attest to their use as an experimental tool, the theory as to the physiological mode of their action is still very problematical. On the other hand, certain steroid hormones, particularly the adrenal cortical and estrogenic hormones, have been shown to exert a
protective action on x-irradiated animals. Thus, a fourfold study presents itself: (1) to study the metabolic effects of x-irradiation on erythrocytes as an example of isolated cells, (2) to determine whether the protective action afforded x-irradiated animals by the adrenal cortex can be interpreted on a cellular basis from a study of the metabolism of x-irradiated red cells, (3) to initiate, at least, studies of the effects of the thyroid on erythrocyte metabolism since steroid hormones are known to influence, either directly or indirectly, thyroid function, and (4) to undertake, of necessity, as the first study the effect of aging in vitro on red cell metabolism.

In order to carry out future permeability studies it was necessary, on the one hand, to obtain large batches of discrete cells, and, on the other hand, to obtain these cells from an organism possessing certain endocrine glands that would lend themselves to total extirpation. The rat appeared to be most suitable for this purpose. The non-nucleated red cell being an isolated unit and possessing many distinguishing attributes, so aptly cataloged by Jacobs (1931), is itself open to the criticism that, in lacking a nucleus, it is an "abnormal" cell, and hence, a "dying" cell. R. S. Lillie (1923) in listing, on general physiological
grounds, those properties which distinguish animate from inanimate matter, stressed the property of metabolism as of prime importance in characterizing living matter. When this property was applied by earlier investigators to the non-nucleated red cell, the then-existing evidence re-enforced the view that the non-nucleated red cell is not the best biological material, notwithstanding the extensive literature in the field of permeability (Davson and Danielli, 1943; Ponder, 1948) and Jacobs' extolling its virtue for research. The following investigators reported little or no oxygen consumption for non-nucleated erythrocytes: Warburg, 1909; Harrop, 1919; Daland and Issacs, 1927; Barron and Harrop, 1928; and Michaelis and Salomon, 1930. Not until Ramsey and Warren (1930, 1932) reinvestigated the subject was a new interpretation made that the oxygen uptake of non-nucleated red cells "is comparable to other resting tissues." This work was inadvertently confirmed by Gonzalez and Angerer (1947) in their search for large quantities of isolated cells which are metabolically sensitive to adreno-cortical insufficiency. These latter workers pointed out the possible sources of error serving to give investigators prior to Ramsey and Warren negative results for the oxygen consumption of non-nucleated red cells. Ponder (1948) suggested
that the respiration of non-nucleated erythrocytes is significant, on absolute grounds, if the mass of inert hemoglobin is subtracted from the total "tissue" weight. For example, assuming that 2.5 ml. of 50% red-cell suspension has a dry weight of 334 mg., with an oxygen consumption of 30.06 cmm. per hour (personal observation), and assuming further, that the inert hemoglobin constitutes 95% (Ponder, 1948) of the cell mass, then the cmm. of oxygen consumed per mg. of dry weight of red cell "protoplasm" per hour ($Q_{O_2}$) is approximately -1.8. When this value is compared to -2.5, -11, -21, -10, -6.5, and $Q_{O_2}$ for resting skeletal muscle, liver, kidney (Ponder, 1948), brain (Crismon and Field, 1940), skin (Walter, Sharlit, and Amersbach, 1945), and sciatic nerve (Gerard, 1930), respectively, it does not appear to be as insignificant as once thought.

It has been shown that following adrenalectomy body water shifts into nucleated (Harrop, 1936; Hegnauer and Robinson, 1936) and likewise into non-nucleated erythrocytes (Gonzalez and Angerer, 1947). Netsky and Jacobs (1941) have demonstrated a decreased permeability of the latter cells to glycerol from low concentrations of Compound E and the amorphous fraction of the adrenal
cortex. Such evidence suggests a relationship between the adrenal, the oxygen consumption, and the state of membrane permeability of the non-nucleated erythrocyte.

Thyroid and adrenocortical activity are closely related in the intact animal as evidenced by the change in adrenocortical function in hypothyroid and hyperthyroid rats (Deane and Greep, 1947; Wallack and Reineke, 1949), and by the lack of the adrenocortical hormone in reducing the efficacy of thyroxin on the oxygen consumption of rats (Hoffman, Hoffman, and Talesnik, 1948). It is very possible that the presence or absence of the thyroid hormone may be a vital factor in the interpretation of adrenocortical function at the cellular level. It is also suggestive to think that, if these cells lend themselves to thyroid studies, they would be of value for investigations concerning the interplay of steroid and non-steroid hormones.

In terms of the general physiology of normal cells and tissues roentgen rays appear to have certain experimentally reproducible effects, such as an increase in permeability to water (Williams, 1923) and to certain solutes (Gasul, 1927), a change in protoplasmic viscosity (Northern and MacVicar, 1940), and an alteration in the metabolism of certain cells (Williams and Sheard,
1932; Penn and Latchford, 1932; Frankenthal and Back, 1944; Back and Frankenthal, 1947; Tahmisian, 1949). Certain hormones like the adrenocortical (Patt, Straube, and Tyree, 1949; Graham, Graham, and Grafeo, 1950) and the estrogenic hormones (Treadwell, Gardner, and Lawrence, 1943; Patt, Straube, and Tyree, 1949), when administered prior to whole body x-irradiation, tend to protect the animal (as measured by the percent survival) against the deleterious effects. On the other hand, the thyroid hormone tends to potentiate these effects (Blount and Smith, 1949). It is apparent, therefore, that roentgen rays may be a useful tool for inducing certain experimentally desirable alterations in red-cell function. However, no quantitatively valid experiments exist as to the effect of roentgen rays on permeability. The most striking experiments tending to support an increase in permeability, as tested by the exosmosis of certain solutes (Lehman and Wels, 1926), were done on red cells. Of course, these experiments are open to the criticism that x-irradiations in themselves upset the internal equilibrium of the cell by releasing ions from a bound state (Heilbrunn and Mazia, 1936). There is, furthermore, no known literature on the effects of x-irradiation on the oxygen consumption of non-nucleated erythrocytes. Studies with high x-ray dosages (500,000 r.
to 2,000,000 r.) have been reported on nucleated erythrocytes of fowl (Frankenthal and Back, 1944; Back and Frankenthal, 1947), and such studies indicate that the nucleus is responsible for one-eighth to one-tenth of the respiration of the intact cell. This tends to substantiate the studies of Hunter (1941) on normal erythrocytes of the same species. Hunter claims that the oxygen consumption of the nucleus represents only a small fraction of that of the intact cell and suggests that the nucleus is not the center of metabolic activity.

The initial desire in these studies was to irradiate the erythrocytes with more clinical (e.g., 8,000 r. as used by Freedman and Dundon, 1947) than academic (e.g., 500,000 r. or 2,000,000 r. as employed by Frankenthal and Back, 1944) dosages of x-rays. On the basis of some early studies on the protoplasmic viscosity of single cells like leukocytes and amoeba (Angerer, 1937) it was suggested that there might be a delayed response in red cells following their exposure to light dosages of x-rays. A similar delay response has been recorded for the Clodoceran, Daphnia, by Obreshkove and King (1932), who found a decrease in the oxygen consumption six days after irradiation of 10,000 r. to 25,000 r. It has been observed that the oxygen consumption of frog skin subjected to 9,000 r. increased
from sixteen to eighteen days and then decreased below the normal value on the thirtieth day (Williams and Sheard, 1932).

Although data exist as to the effect of aging in vitro on the oxygen consumption of certain tissues (Walter, Sharlit, and Amersbach, 1945), there is no literature on the metabolism of aging erythrocytes in vitro. Thus in order to study delayed effects of x-irradiation as it affects oxygen consumption, it became necessary to study first the effect of aging in vitro on the oxygen consumption of erythrocytes.

These studies were undertaken with the view of continuing the investigations initiated by Gonzalez and Angerer (1947) on the role of the adrenal cortex and of instituting investigations on the role of the thyroid on the metabolism of non-nucleated red cells. These studies may be specifically classified under the following headings: (1) the effect of aging in vitro on the metabolism of erythrocytes; (2) the effect of more clinical dosages of x-irradiation on red-cell metabolism; (3) the role of the adrenal cortex in antagonizing, or in protecting, against any alteration in the metabolism of red cells after exposure to x-irradiations; and (4) the effect of thyroid hormone on the metabolism of non-
nucleated erythrocytes. The latter study was of interest because of the metabolic interrelation known to exist between the adrenal cortex and the thyroid.
MATERIAL

Theory of the Warburg apparatus. There are essentially three types of apparatus (Dixon, 1943) used for measuring the oxygen consumption of tissue: (a) the "differential" type in which the pressure and volume change simultaneously due to a rigidly closed system; (b) the "constant pressure" type in which any pressure change is compensated by a reciprocal change in volume, effected by means of a moveable fluid column serving as a piston; and (c) the "constant volume" type which now will be discussed at greater length.

Given a stoppered flask with a horizontal tube attached to it (Figure 1), the system is closed by placing a drop of fluid in the horizontal tube. Should living tissue be placed in this closed system along with some means for absorbing the carbon dioxide produced, the fluid (index) drop would move towards the flask as the oxygen pressure within the flask is decreased, due to the oxygen consumption of the respiring tissue. The amount of linear, volume displacement of the index drop is an indirect measure of the oxygen uptake.

In the "constant volume" type of respirometer the gas-fluid interface of the fluid column nearest (proximal) to the respiration flask is kept on a fixed
FIGURE 1. THE SIMPLE PRINCIPLE OF THE WARBURG MANOMETER.

FIGURE 2. RESPIRATION FLASK IN CONJUNCTION WITH U-TUBE AND RESERVOIR.
(index) mark at an arbitrary, but known distance, from the respiration flask. Of course, to maintain the proximal gas-liquid interface of the fluid column on the index mark of the capillary tube, while the gaseous pressure is decreasing in the closed system, necessitates the incorporation of a fluid reservoir to supply varying external pressures on the fluid column. A ruled scale is superposed on the opposite end of the liquid column in order to measure any displacement of the distal air-liquid interface which results from the difference in the pressure gradient between the atmospheric air and the respirometric gas. This gradient across the interposed fluid column is the resultant of a pressure differential between the atmospheric air (open, distal end) and the respiration chamber (closed, proximal end). To meet this demand, the straight tube (Figure 1) is replaced by an U-tube (Figure 2) at the bend of which is attached a fluid reservoir. Through this fluid reservoir any externally applied pressure may be reflected on the fluid column of the U-tube. The external pressure is applied through a mechanical, screw device. The left hand limb is set at the index mark and any change in position of the proximal gas-fluid interface of the right hand limb is read at known intervals of time. Thus the rate of oxygen consumption may be determined. This
is the underlying principle of the Barcroft-Warburg apparatus.

**Description of the Barcroft-Warburg apparatus.**

The "constant volume" type of respirometer was originally developed by Barcroft but, because of its extensive use by O. Warburg, it is called the Warburg manometer or respirometer. The respirometer consists of an U-tube of a narrow bore (capillary) with the vertical limbs approximately 30 cm. long and graduated to permit readings from 0 to 300 mm. (Figure 3). A rubber reservoir is attached to the curved portion between the two limbs to allow fluid of a known density (Dixon, 1943; Umbreit, Burris, and Stauffer, 1945) to be moved up or down the graduated limbs by means of a screw, clamp device which serves to increase or decrease the pressure applied to the fluid reservoir. One end of the tube is open to the atmospheric pressure, while the other end is connected by means of a ground glass joint to the respiration flask. This flask contains the cells or tissue whose metabolism is to be studied. The proximal (closed) end of the tube contains a stopcock above the level of the respiration flask to permit a closed or open system as desired.

The respiration flasks have a volume of between 15 and 18 ml; attached to each flask are two side arms which may be used for substrates, drugs, and so on.
FIGURE 3. WARBURG MANOMETER.
Figure 4. Constant temperature bath with manometers.
Located in the main chamber of the flask is a small central well into which is placed a strong alkali for the absorption of carbon dioxide. The manometers were used in conjunction with a constant-temperature (37.5 ± 0.01°C.) water bath (Figure 4) and were shaken at eighty oscillations per minute through a distance of 3.9 cm.

**Calibration of the Barcroft-Warburg apparatus.**

To determine the amount of gas absorbed (oxygen consumption) from the observed readings of the scale on the distal capillary limb, it is necessary to know the gaseous volume of the closed end of the respirometer to the stationery proximal gas-fluid interface. This calibration, when oxygen is the gaseous atmosphere in the closed system, is called the flask constant for oxygen ($k_{O_2}$). Thus each Warburg manometer has its own flask constant for a given gaseous atmosphere. To calculate the flask constant it is necessary, for manipulative consideration, to calibrate each manometer on the basis of its having three parts: 1) the manometric side arm containing the stopcock and male joint, 2) the scaled proximal limb of the capillary U-tube, and 3) the respiration chamber. The volumetric determination of each of these specific parts follows. The gravimetric, mercury method was used for the calibration of the volume of the manometric system (Dixon, 1943). An arbitrarily
predetermined mark is fixed (etched) on the manometric side arm which extends from the proximal limb of the capillary U-tube. The calibration of the manometer tube from the fixed mark to some predetermined level of the ruled proximal stem of the capillary U-tube is considered first. Sufficient mercury is introduced into the opened male joint, whose female counterpart forms the neck of the respiration flask, to completely fill all portions of the limb carrying the respiration flask, as well as the closed stopcock. This mercury column is allowed to run for a short distance into the ruled, proximal limb of the U-shaped capillary. The air-mercury interface coinciding with the ruled manometric scale is read. The procedure is repeated with the mercury column allowed to run to a different point on the manometric scale. This permits the calculation of the volume per scale division along the graduated proximal limb. The volume of the gaseous space between the etched mark and a known level (e.g., 250 mm.) on the proximal limb is readily calculated by determining the difference between its total volume and the volume occupied by the graduated portion of the proximal limb. There remains a third calibration, that is, the volume of the respirometric flask and of the stem, containing the male joint, up to the etched mark. With the flask side-arm stoppers
firmly in place, the flask is filled with mercury of such a volume as to cause its meniscus to coincide with the etched mark on the manometer stem when it is tightly coupled at the ground-glass joint.

In summary there are nine determinations made as follows: three determinations for the respiration flask; three for the manometric stem; and three for the manometric capillary limb. For each determination the mass and temperature of the mercury used in ascertaining the volume of the part of the apparatus in question was obtained. Knowing the temperature of the mercury at the time of its use in determining the volume of the specific part of a given manometer, the mass, and the density, the volume is readily calculated. The volumes for the respective parts of the manometer are averaged. From these data the flask constant for an oxygen atmosphere \( k_{O_2} \) is readily calculated after allowance is made for the encroachment on the original volume of the space occupied by known volumes of bathing solution for the tissue, of substrate, and of alkali. The volumes occupied by the tissue to be placed in the system as well as by the gases dissolved in the fluid and tissue (Henry's Law) must be considered.

A protocol showing the calibration of a
respirometer follows:

Calibration values for apparatus #1.

Etched mark to 300 mm. mark.
Mercury 43.7561
Tare-35.8381
7.9180 grams

Etched mark to 228 mm. mark.
Mercury 43.6814
Tare-34.2103
9.4771 grams

Grams of mercury per division.
9.4771-7.9180 = 0.022272 grams
70 divisions =

Cmm. per division.
0.022272 = 1.64 cmm.
13.5291 (density of mercury at 27°C.)

Volume from etched mark to 250 mm. mark.
1,000 x 1.64 x 50 + 7.9180 = 0.6672 ml.

Volume for flask #1.
Mercury 299.3028
Tare- 33.6284
265.6044 grams

265.6044 = 19.63 ml.
13.536 (density of mercury at 24.4°C.)

Total volume to 250 mm. reading on scale.
Flask volume 19.63
Stem volume + 0.67
20.30 ml.

Calculation of the flask constant (k\textsubscript{O\textsubscript{2}}). In the equation to be presented, the following symbols or letters are presented:

X is the amount of gas absorbed in cmm. at S.T.P.
h is the reading of the manometer.

$V_g$ is the volume of the gas space in the vessel and manometer to the 250 mm. mark.

$V_f$ is the volume of the liquid in the vessel.

T is the absolute temperature of the water bath.

P is the initial pressure in the vessel.

$P_o$ is the normal pressure in mm. of the Brodie solution (Dixon, 1945).

If D is the density of the Brodie solution, then $P_o$ is equal to $760 \times \frac{13.6}{D}$.

p is the vapor pressure of the water at temperature T.

a = the solubility of the oxygen in the liquid in the vessel (cmm. of gas at S.T.P.) dissolved in cmm. of the liquid when in equilibrium with a partial pressure of the gas equal to $P_o$.

From the above information the following will hold true:

$V_g \frac{273 (P-p)}{T P_o}$ is equal to the initial volume of gas in the gas space.

$V_f \frac{a (P-p)}{P_o}$ gives the initial volume of dissolved gas.

$V_g \frac{273 (P-p)}{T P_o} + V_f \frac{a (P-p)}{P_o}$ equals the total initial gas.
\[
\frac{V_f a (P-p-h)}{P_0} + \frac{V_g 273 (P-p-h)}{T P_0}
\]
represents the final gas.

\[X = \text{the initial gas volume minus the final gas volume, e.g.,}\]

\[h = \frac{V_f a + V_g 273/T}{P_0} \quad \text{If } k_{O_2} \quad \text{is } \frac{V_f a - V_g 273/T}{P_0}, \text{ then }\]

\[X = h \cdot k_{O_2}.
\]

**Reading of the manometer.** The oxygen consumption of respiring cells or tissue is measured indirectly by reading the rate of displacement of the distal air/fluid interface. The rate of displacement is a function of the rate of absorption of oxygen by the tissue and of carbon dioxide by the alkali. Since the oxygen is absorbed, the readings for oxygen consumption are expressed as negative values. These are read in mm. but the final quantity of gas absorbed is expressed after calculation as cmm. of dry gas at standard temperature and pressure (S.T.P.). The gas-fluid interface of the proximal end of the liquid column in the capillary tube is maintained at a constant level (e.g., 250 mm. mark) during an experimental run when readings are taken. The opposite, or distal, capillary limb, which is read, will reflect the pressure changes due to the uptake of the oxygen. The difference between the initial reading
(beginning of the experimental run) and the experimental reading (end of each subsequent 15-minute interval) is recorded. Since the atmospheric pressure and/or temperature may fluctuate during an experimental run, another Warburg manometer, prepared like any other manometer in a bank except that it lacks living tissue, is used as a thermobarometer. The difference between the readings of the initial and final readings of this thermobarometer, which are taken simultaneously with the readings for the experimental manometers, is then added to or subtracted from the appropriate readings of the latter manometers, depending on whether a decrease or increase, respectively, is observed for the thermobarometric reading. This corrected reading, which is multiplied by the flask constant \( k_0 \) for the specific manometer in question, gives the cmm. of gas absorbed at S.T.P.

**Oxygen diffusion.** Dixon and Elliot (1930) have shown that there is a definite relationship between the amount of tissue in the respiration flask and the rate of shaking of the apparatus on the apparent value of the oxygen consumption. If the rate of shaking varies the apparent oxygen consumption, then the rate of diffusion of oxygen through the liquid phase of the system is related, within limits, to the rate of shaking.
On the other hand, if the amount of tissue is large, the oxygen consumption will be determined by the amount of oxygen available to the tissue. Both the amount of tissue and the rate of shaking have a definite limiting value for which the true respiration of the cells is measured. This can only be determined by trial experimental runs of the tissue under consideration. For the conditions of these experiments eighty translatory oscillations of the manometers per minute through an amplitude of 3.9 cm. was found to be adequate.

Carbon dioxide absorption. Dixon and Elliot (1930) demonstrated on the Barcroft apparatus the effect of inadequate carbon dioxide absorption. It is essential that the alkali placed in the flask absorb instantaneously the carbon dioxide in order to maintain a theoretically zero pressure of the carbon dioxide in the gaseous phase. If this is not so, the manometer readings will not represent the true oxygen uptake.

For the Barcroft apparatus alkali papers are recommended. The alkali-soaked filter paper is placed in the central well and projects above for approximately 3 cm. Since there is more surface present in the central well of the respiration flask of a Warburg manometer, it has been found that alkali papers are not necessary. This is particularly so when measuring
erythrocytes which have a small oxygen uptake and subsequently a small carbon dioxide output. A 10% sodium hydroxide solution was found to be more than adequate to act as a carbon dioxide absorber for the system in question.

X-ray apparatus and equipment. Two x-ray machines designed for clinical work were used; one purchased from the General Electric X-ray Corporation and the other from the Westinghouse Electric Corporation. The former was used for the studies involving different dosages of x-irradiation. The blood samples, submerged in a 15 cm. rice phantom at a distance of 19 cm. from the source of unfiltered x-irradiations (250 k.v.p. and 15 ma.) were subjected to exposures at the rate of 625 roentgens per minute as measured by a Victoreen ion chamber. For the studies involving the adreno-cortical extract, the Westinghouse machine was used. In this case no rice phantom was necessary and the blood samples, placed at a distance of 14 cm. from the source of the irradiation (0.5 mm. copper and 1.0 aluminum filters, and at 250 k.v.p. and 15 ma.) were exposed at the rate of 1250 roentgens per minute for twelve minutes. Since $10^5$ roentgens raises tissue temperature approximately 0.25°C. and this is felt to be inadequate to explain the biological effects of ionizing radiations.
(Lea, 1947), no attempt was made to register temperature changes during x-irradiation.
PROCEDURE

Preparation of equipment. All glassware (e.g., respiration flasks, syringes, centrifuge tubes, and the like) was soaked overnight in solutions of green soap and trisodium phosphate. It was then rinsed in tap water and subsequently in pyrex distilled water, and dried at 110°C. just prior to use. Upon removal from the drying oven, that part of the glassware to be in contact with blood was kept as sterile as possible until used.

Preparation of animals. Male albino rats (100 to 150 grams) were purchased from the Harlan Small Animal Laboratory, Cumberland, Indiana and were maintained under laboratory conditions at least one week prior to use. During this period they were permitted to feed ad libitum on Purina Dog Chow and tap water. For the particular studies involving adrenal cortical extract, 0.05 ml. of Upjohn's Adrenal Cortical Extract in saline and 10% alcohol was injected subcutaneously once per day per rat for 10 to 15 days prior to isolation and subsequent irradiation. Food was removed from all animals 12 to 24 hours prior to the withdrawal of their blood in order to render the animal in a "basal" (postabsorptive) state.
For those studies on Man, patients of both sexes were obtained from The Ohio State University Hospital and from The Columbus State School. They were divided, on the basis of their clinical diagnosis (primarily based on blood protein-bound iodine), into four alphabetic groups as follows: (A) patients with no indication of thyroid disturbances (euthyroid), (B) untreated hyperthyroid patients, (C) hyperthyroid patients treated with 10 drops of Lugol's solution 3 times daily for 14 to 21 days (not the same patients in group B), and (D) untreated hypothyroid patients. No attempt was made to place the patients under "basal" conditions before taking a blood sample.

Preparation of the tissue. Using 5 ml. syringes, 22 gauge needles, and heparin as an anticoagulant, blood was drawn from the rats by means of cardiac punctures. An average of 2 to 3 ml. of blood was obtained from each rat. The blood from a large number of rats (20 to 43) was pooled. Fifty ml. of blood was withdrawn by venous puncture from each patient for individual study. In both cases the red cells were then centrifuged to constant volume. The centrifuge head had a 6 inch radius and a rotational velocity of 1500 r.p.m. when loaded. The plasma and the "buffy coat" were removed by means of a capillary-pipette suction apparatus. The erythrocytes
were washed twice with Krebs-phosphate-Ringer (Krebs) solution (Krebs and Henseleit, 1932) and resuspended to their own volume (50% suspension) in Krebs solution. In the studies with x-irradiation the pooled red-cell suspensions were divided so that a constant volume of the suspension was always exposed to x-rays. For example, 50 ml. of the red cell suspension was used for studies involving the effect of different roentgen dosages; 20 ml., for studies treating of the adrenal cortical extract; and the remainder of the red-cell pools for control studies. When the experiments were not terminated on the day the blood was drawn, the red cell suspension was stored at 2° to 8°C. It was later returned to room temperature (25° to 30°C.) for withdrawal of red cell samples preparatory to the determination of their oxygen consumption and of their count.

A typical experimental run. After preparation of the glassware and water bath, the Krebs solution and the alkali were oxygenated. The red cell suspension was gently shaken to assure an equal distribution of the contained red cells. A red cell count was taken by means of a Spencer Bright Line Haemocytometer. On earlier red-cell pools a bacteria check was made by means of the dry smear technique using a methylene blue
as the stain. (Kolmer, 1944).

Into the central well of each respiration flask was placed 0.2 ml. of 10% sodium hydroxide solution. The blood was then oxygenated for 2 to 4 minutes to a bright scarlet color (arterial character), and 2.5 ml. aliquots were taken, by means of a fairly large-mouthed pipette, and placed, respectively, into from 3 to 6 of the respiration flasks. The respirometers were flushed with pure oxygen for 10 minutes after which time they were allowed to equilibrate for 10 minutes in the water bath. Upon equilibration the fluid column was set at a previously determined value on the proximal glass limb of the manometer. The taps were closed and the initial reading was taken from the distal limb of the manometer. Thereafter, the manometric shaking was stopped at the end of a predetermined interval of time to record the readings. All readings were observed for the actual time indicated and were not extrapolated from some lesser value.

**Calculation of oxygen consumption.** In order to standardize the readings for making comparisons of metabolic studies, it is necessary to have a common means of expression. This standard is the $Q_{O_2}$, or $\text{cmm.}$ of oxygen consumed (absorbed) per hour per mg. of dry weight of tissue at S.T.P. ($Q_{O_2} = \frac{-\text{reading in mm.}/\text{hr}}{(\text{mg. dry weight})} k_{O_2}$
Since the thyroid studies involved measurements either at the time of isolation of the red cells or during the following day, and since the previous work had shown no significant difference in $Q_{O_2}$ values of these cells under these circumstances, a dry weight determination was made of three red-cell suspensions. The average of these three values was taken to be the dry weight for all subsequent calculations treating of erythrocytes.

In view of the experiments on aging of red cells in vitro which were carried on over a longer period of time, it was decided that a truer $Q_{O_2}$ value would be attained for the irradiation experiments were oxygen consumption values based on the number of intact red cells. Using one red-cell pool as a standard, a red cell count was taken and 2.5 ml. aliquots were placed in a 110°C oven to dry overnight. Since 50% of the suspension was due to Krebs solution, 1.25 ml. samples of this solution were also dried overnight. The difference in the dry weights between the red cell suspension and the Krebs solution gave the dry weight for a suspension of red cells at that particular count. The dry weights of different red-cell pools, as well as of the same pool over a period of time, were then calculated on the basis of their red cell counts as compared to the pool
which had been actually dried overnight in the oven.

Dry weight of new pool:

\[
\frac{(\text{count of new pool})}{\text{count of original pool}} \times (\text{dry weight of original pool})
\]

Dry weight of pool on the fourth day:

\[
\frac{(\text{cell count on fourth day})}{\text{cell count on zero day}} \times (\text{dry weight on zero day})
\]

The total oxygen consumption of the 2.5 ml. suspension is expressed in the numerator of the \( \dot{Q} O_2 \) equation. Thus the oxygen consumption (cmm.) per billion red cells is calculated as follows:

\[
\frac{(\text{readings in mm./hour})}{(2500 \text{ cmm.})} \times (10^9 \text{ red cells}) \times (\text{red cell count/ommm.})
\]

The original dry weight determinations are presented. The red cell count was 5,520,000 per cmm.

Dry weight of 2.5 ml. suspension of red cells.

<table>
<thead>
<tr>
<th>Dry weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3460</td>
</tr>
<tr>
<td>0.3463</td>
</tr>
<tr>
<td>0.3439</td>
</tr>
<tr>
<td>0.3550</td>
</tr>
<tr>
<td>0.3583</td>
</tr>
<tr>
<td>0.3476</td>
</tr>
</tbody>
</table>

\[ \frac{2.0771}{6} \text{ grams} \]

The average dry weight is \( \frac{2.0771}{6} \), or 0.346 grams.

Dry weight of 1.25 ml. Krebs solution.

<table>
<thead>
<tr>
<th>Dry weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0123</td>
</tr>
<tr>
<td>0.0122</td>
</tr>
<tr>
<td>0.0125</td>
</tr>
</tbody>
</table>

\[ 0.0370 \]

The average dry weight is \( \frac{0.0370}{3} \) or, 0.0123 grams.
The average dry weight for 5,520,000 red cells/mm$^3$ is 0.346 - 0.0123 or 0.334 grams/2.5 ml. suspension.
RESULTS

The effect of aging in vitro on the oxygen consumption of rat erythrocytes. A total of 24 red-cell pools was studied for various intervals of time between 2 and 21 days. Erythrocyte counts were taken in 17 of these red-cell pools each day that an experimental run was made. At first the counts were taken from the pools, but later, they were taken from the respiration flasks at the end of the run, since it was felt this would give more accurate values. In general, there was no significant difference between the oxygen consumption on the day of withdrawal and that measured after overnight storage (2°C to 8°C) of the different pools. The mean \( Q_{O_2} \) and the average cmm. of oxygen consumed per billion red cells on zero day are 0.09 and 2.26, respectively.

No oxygen consumption was observable for the Krebs solution whether it was taken from a fresh or from a one-day-old medium from which the red cells had been removed. Likewise, there was no observable effect on the oxygen consumption of a fresh suspension upon the addition of an aged medium, nor of an aged suspension upon the addition of a fresh medium.

Although the quantitative values for the oxygen consumption of the different red-cell pools vary rather
greatly, the general nature of the curves representing this oxygen consumption plotted as function of time gave relatively similar slopes. Experiment eleven is presented as indicating the results obtained from a typical pool, since its values closely approximate the average of all pools. The $Q_{O_2}$ values for this pool are presented in two ways: (1) those based on a standard dry weight (334 mg. per 2.5 ml. of a 50% red cell suspension) which was utilized for the $Q_{O_2}$ determinations on each day, and (2) those based on a dry weight which was calculated from the erythrocyte count on the day that each measurement was made (Table 1 and Figure 5).

The effect of x-irradiation on the oxygen consumption of rat erythrocytes in vitro. A total of thirteen red-cell pools (32 to 43 rats per pool) was studied over various intervals of time (1 to 4 days) after exposure to x-irradiation of 10,000 r., 20,000 r. and 30,000 r. dosages. Expressing the oxygen consumption in terms of the number of intact erythrocytes, all irradiated red-cell pools, with one exception, show a greater oxygen consumption than the paired controls over the same interval of time. A summary of the data, expressed in terms of the mean value for the red cell count and for the oxygen consumed per billion red cells for the
Table 1. The effect of aging in vitro on a red cell suspension. The data are from a typical suspension.

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>6</th>
<th>***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cells/ccm. (x10^4)</td>
<td>560</td>
<td>513</td>
<td>532</td>
<td>447</td>
<td>-20%</td>
</tr>
<tr>
<td>Cmm. ( Q_0/10^9 ) red cells</td>
<td>2.51</td>
<td>2.59</td>
<td>1.88</td>
<td>1.75</td>
<td>-30</td>
</tr>
<tr>
<td>* ( Q_0 )</td>
<td>-0.104</td>
<td>-0.107</td>
<td>-0.078</td>
<td>-0.073</td>
<td>-30</td>
</tr>
<tr>
<td>** ( Q_0 )</td>
<td>-0.104</td>
<td>-0.098</td>
<td>-0.074</td>
<td>-0.051</td>
<td>-50</td>
</tr>
</tbody>
</table>

* Based on dry weight determined from cell count.

** Based on actual dry weight determination of samples from a so-called "standard pool." The same weight was employed for each day \( Q_0 \) value.

*** The percent difference in comparing the sixth day with zero day.
FIGURE 5. THE RELATION BETWEEN THE RED CELL COUNT, THE OXYGEN CONSUMPTION PER BILLION RED CELLS, THE $Q_02$ OF A RED CELL SUSPENSION, AND TIME.

$0 = \text{CMM. OXYGEN CONSUMED PER } 10^9 \text{ RED CELLS}$

$\bullet = \text{ERYTHROCYTE COUNT PER CMM.}$

$\Delta = Q_{02} \text{ BASED ON INITIAL DRY WEIGHT.}$

$\Delta = Q_{02} \text{ BASED ON CELL COUNTS.}$
different roentgen dosages is presented in Table 2. The oxygen consumption of the irradiated red-cell pools on the first day is equal to, or slightly exceeds, that which is obtained on the day of isolation, that is, before x-irradiation. The oxygen consumption for red cells exposed to the two lower dosages (10,000 r. and 20,000 r.) on the second and third day decline paralleling the controls but at a higher level. The 30,000 r. dosage causes no decline in oxygen consumption over the four day period studied (Figure 6). The degree of change in the oxygen consumption appears to be a function of time and of dosage (Figure 7). A comparison of the x-irradiated red-cell pools with their controls shows no statistical significance (Student's method) with regards to time.

Although there is a greater decrease in the number of red cells with time in the irradiated red-cell suspensions than in their controls, the mean oxygen consumption of the cell suspensions, both irradiated and control, is similar. This is illustrated in Figure 8 which shows the mean values for the oxygen consumption of red-cell pools exposed to 30,000 r. The decrement in erythrocyte count with time is similar for the 10,000 r. and 20,000 r. dosages, but is less than for the 30,000 r. dosages (Figure 7).
Table 2. Summary of the mean oxygen consumption and mean cell count of rat erythrocytes subjected to x-irradiation in vitro.

<table>
<thead>
<tr>
<th>Dosage of x-irradiation</th>
<th>No. of pools</th>
<th>Cmm. $O_2/10^9$ red cells control x-ray %difference</th>
<th>Red cell count/cmm. ($x10^4$) control x-ray %difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 r.</td>
<td>3</td>
<td>1.95 2.50 + 28</td>
<td>539 478 - 11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.06 2.50 + 34</td>
<td>448 526 - 5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.56 2.00 + 30</td>
<td>526 425 - 19</td>
</tr>
<tr>
<td>20,000 r.</td>
<td>2</td>
<td>1.99 2.54 + 27</td>
<td>592 514 - 9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.62 2.06 + 27</td>
<td>559 479 - 14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.16 1.69 + 45</td>
<td>604 465 - 23</td>
</tr>
<tr>
<td>30,000 r.</td>
<td>7</td>
<td>2.24</td>
<td>534</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.91 2.31 + 20</td>
<td>633 519 - 18</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.63 1.96 + 20</td>
<td>552 456 - 17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.65 2.34 + 41</td>
<td>556 369 - 31</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.37 2.25 + 64</td>
<td>563 288 - 52</td>
</tr>
</tbody>
</table>
FIGURE 6. THE EFFECT OF X-IRRADIATION AND TIME ON THE OXYGEN CONSUMPTION OF RAT ERYTHROCYTES IN VITRO.

○ = CONTROL

◇ = X-IRRADIATED
FIGURE 7. THE EFFECT OF X-IRRADIATION AND TIME ON RAT ERYTHROCYTE SUSPENSIONS.

○ = FIRST DAY
○ = SECOND DAY
○ = THIRD DAY
FIGURE 8. THE EFFECT OF X-IRRADIATION (30,000 R.) ON THE MEAN OXYGEN CONSUMPTION AND MEAN CELL COUNT OF RAT ERYTHROCYTE SUSPENSIONS.

○ = CONTROL
0 = X-IRRADIATED
The effect on the oxygen consumption of x-irradiated erythrocytes from normal rats previously injected with adrenal cortical extract. A total of 10 pools (18 to 25 rats per pool) were studied. Half of the pools consisted of cells from normal animals injected with whole adrenal cortical extract (ACE) and the other half from normal untreated animals. Each pool was paired and one of the pair was x-irradiated with 15,000 r. immediately after isolation (i.e., within one hour). The oxygen consumption was measured as soon as possible after x-irradiation. The elapsed time from the end of the exposure to the specified roentgen dosage to the immersion of the respirometers in the water bath was approximately 45 minutes and 21 hours respectively. In comparing the oxygen consumed per billion red cells for the ACE x-irradiated red-cell pools with the ACE non-irradiated pools (5 pools), the former show a - 28% to + 12% variation immediately after x-irradiation and a 0% to + 57% (4 pools) after overnight storage. A comparison of x-irradiated with non-irradiated red-cell pools (5 pools) from normal rats with regards to time showed a + 7% to a + 30% variation in the oxygen consumption when studied immediately after x-irradiation and a - 6% to + 39% variation (5 pools), after overnight
storage. A comparison of the data for the mean oxygen consumption (per billion cells) of x-irradiated and of paired, non-irradiated red-cell pools (control), is presented in Table 3. The relationship of this study to the previous study (10,000, 20,000, and 30,000 roentgen dosages) is presented in Figure 9.

The oxygen consumption of erythrocytes from euthyroid, hyperthyroid, iodine-treated hyperthyroid, and hypothyroid patients. The oxygen consumption of erythrocytes from 39 patients were studied; each patient constitutes one experiment. The oxygen consumption of non-irradiated cells was studied both immediately after their preparation, by use of the Fenn technique, and after overnight storage in the refrigerator, by use of the Warburg technique. No significant difference in their mean $Q_0$ values was noted for either of these two periods of time. Eventually, the Warburg technique was used to study not only the "euthyroid" cells but also cells obtained from various "thyroid patients."

The data, expressed as mean $Q_0$ are summarized in Table 4. When compared with the euthyroid group (A), the untreated hyperthyroid group (B) is statistically significant ($P < 0.04$), whereas, the iodine-treated
Table 3. The effect on the oxygen consumption of x-irradiated erythrocytes from normal rats previously injected with adrenal cortical extract.

<table>
<thead>
<tr>
<th>Time after x-ray pools</th>
<th>Mean oxygen consumed / 10^9 red cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of irradiated pools</td>
<td>Normal</td>
</tr>
<tr>
<td>1 hour</td>
<td>3</td>
<td>2.23</td>
</tr>
<tr>
<td>overnight storage</td>
<td>5</td>
<td>1.74</td>
</tr>
</tbody>
</table>
Figure 9. Summary of the oxygen consumption of rat erythrocytes under various conditions.
hyperthyroid (C) and hypothyroid (D) groups are not
(P < 0.50 and P < 0.20, respectively). All other
combinations of the various groups are not statistically
significant with the exception of a comparison between
the respiration of red cells from the hypothyroid (D)
and hyperthyroid (B) patients (P < 0.01). Figure 10 shows
the mean $Q_{O_2}$ values for the respective "thyroid" groups,
as listed in Table 4, plotted as functions of the
respective mean plasma protein-bound iodine concentration.
This curve is based on 26 experiments. The justification
and statistical evaluation for a curve of exponential
form is to be found in the appendix (pages 63 and 64).
Table 4. Summary of the mean $Q_{O_2}$ values of red cells from euthyroid (A), hyperthyroid (B), iodine-treated hyperthyroid (C), and hypothyroid (D) patients.

<table>
<thead>
<tr>
<th>Patient groups*</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Mean PBI (μgm.%)</td>
<td>4.3</td>
<td>8.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Mean $Q_{O_2}$</td>
<td>0.013</td>
<td>0.025</td>
<td>0.016</td>
<td>0.011</td>
</tr>
<tr>
<td>Per cent difference</td>
<td>+92</td>
<td>+23</td>
<td>-15</td>
<td></td>
</tr>
<tr>
<td>Standard deviation (±)</td>
<td>0.003</td>
<td>0.014</td>
<td>0.012</td>
<td>0.031</td>
</tr>
<tr>
<td>Standard error (±)</td>
<td>0.001</td>
<td>0.005</td>
<td>0.004</td>
<td>0.010</td>
</tr>
<tr>
<td>&quot;t&quot; value</td>
<td>2.37</td>
<td>0.75</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Significant (P&lt;0.05)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

*See method for characterization of groups.

"t" = Ratio of statistical comparison of the difference between the euthyroid mean $Q_{O_2}$ value and each of the other groups/√(E respective standard errors)^2.
FIGURE 10. THE OXYGEN CONSUMPTION OF HUMAN ERYTHROCYTES AS A FUNCTION OF THYROID ACTIVITY.
A = EUTHYROID
B = HYPERTHYROID
C = IODINE-TREATED HYPERTHYROID
D = HYPOTHYROID
DISCUSSION

On the basis of these results and those of other investigators (Harrop, 1919; Michaelis and Salomon, 1930; Ramsey and Warren, 1930, 1932; Gonzalez and Angerer, 1947), it is apparent that the consistent respiration of a red cell suspension is due primarily to intact erythrocytes. The generally negligible decrease in the oxygen consumption after overnight, cold storage has been observed by other investigators (Michaelis and Salomon, 1930; Ramsey and Warren, 1930; Tipton, 1933). Because of the manner in which the values for oxygen consumption of erythrocytes are expressed in the literature and, also, because of the great variability in the manner of preparation and of concentration of the suspension of the red cells for study, a purely quantitative nature comparison of the results on metabolism of red cells of the same or different species is out of the question. In a previous study (Gonzalez and Angerer, 1947) from this laboratory, in which essentially the same technique was employed as used here, the mean $Q_{O_2}$ value of freshly-drawn rat red-cells was reported as - 0.15. This value is higher than that observed in this study (- 0.09). At the present time we have no adequate explanation for this difference. By
making certain assumptions on the basis of our work, the \( Q_{O_2} \) of human erythrocytes as reported by Ramsey and Warren (1932) on a wet weight basis was calculated to a dry weight basis, and gives an approximate \( Q_{O_2} \) value of -0.02 as compared to -0.013 for "normal" patients (Table 4).

If the data from Table 1 are plotted as functions of time (Figure 5), it is apparent that the method of expressing the oxygen consumption from measurements over a long period may be pertinent for the interpretation of data. This is particularly emphasized in Figure 8. In expressing \( Q_{O_2} \) value it is necessary that the dry weight utilized in the calculations reflect the number of intact erythrocytes in the suspension. When this is done, the \( Q_{O_2} \) values and the oxygen consumed per billion red cells are in close agreement, that is, both exhibit a 30% decrease in oxygen consumption on the sixth day (Table 1). It has been shown that no respiration exists after hemolysis (Harrop, 1919; Michaelis and Salomon, 1932) and, also, that the burst of "respiration" reported at the time of hemolysis (Ramsey and Warren, 1930) is apparently due to a plasma component and not to normal protoplasmic metabolism (Ramsey and Warren, 1934). The decrease in oxygen consumption observed on aging must be strictly proportional to the decrease in red cells,
assuming that the decrease in oxygen consumption is directly proportional to the number of intact erythrocytes in suspension and that a given erythrocyte respires at a constant rate. It may be that this is not the case (Table 1) since the per cent decrease in the red cell count (- 20%) tends to be less than the per cent decrease in the oxygen consumption (- 30%). Although this difference falls within the range of experimental error, the mean values of all red-cell pools show the same tendency. It appears, therefore, that over long periods of time the decrease in oxygen consumption of a suspension may involve at least two factors: the decrease in the number of erythrocytes and the decrease in the respiration of each cell.

It has been reported (Walter and Sharlit, 1945) that certain tissues (kidney, liver, and skin) kept at 4°C. over a period of seven days exhibit a 78% to 90% decrease in their $Q_{O_2}$ values. The erythrocytes show the same tendency but to a lesser degree (- 30%). However, one of the factors accounting for the large discrepancy in the per cent decrease from a comparative viewpoint may be due to determination of $Q_{O_2}$ values irrespective of the number of intact functioning cells.

Stored non-nucleated erythrocytes show fragility, dimensional, and chemical changes with time (Maizels
and Whittaker, 1939; Maizels, 1943; Gillespie, 1943; Rapoport, 1937; and others). From a series of studies on nucleated erythrocytes (Hunter, 1939, 1941, 1947, Hunter and Banfield, 1941), it was suggested that the maintenance of membrane permeability does not depend upon the energy derived from metabolic processes in the cell. However, the studies of Ponder (1949) on non-nucleated erythrocytes are particularly impressive. Human red cells suspended in isotonic NaCl and stored at 4°C, show a continuous loss of potassium over a period of six days, at which time, this loss amounts to 35%. That a relationship exists between potassium permeability and oxidative metabolism is suggested by the corresponding 30% decrease in the oxygen consumption of the rat erythrocyte over the same period of time.

Since there is no literature on the effects of x-irradiation on the oxygen consumption of non-nucleated erythrocytes, no comparison can be made. However, Frankenthal and Back (1944) have observed on nucleated fowl erythrocytes subjected to roentgen dosages of 50,000 to 2,000,000 an initial transient stimulation in oxygen consumption (greatest value occurring 30 minutes after irradiation) which was followed by a decrease in oxygen consumption. This they correlated
with hemolysis. Their observations were limited to a period of ninety minutes after x-irradiation, and no effect was observed with dosages under 500,000 r. These results are expressed in microliters of oxygen consumed per ml. of erythrocyte suspension. The data presented on rat erythrocytes (Figure 8) indicate a true irradiation effect has been masked, at least from relatively low dosages of irradiations. Also, the lack of an observed effect on the nucleated erythrocytes with dosages under 500,000 r. may be due to lack of consideration of the number of intact cells. Although the data are as yet incomplete for definite conclusions as to the effect of 15,000 r., it appears that non-nucleated erythrocytes tend to show an increased oxygen consumption immediately after x-irradiation as compared to the paired controls.

It appears (Figures 7 and 9) that the respiratory response in red cells is dependent not only upon the time at which the oxygen consumption is measured but also upon the roentgen dosage. During the first and second days the mean per cent variation in the $Q_{O_2}$ values from the control is similar for any given sample of red cells exposed to a given roentgen dosage. However, in comparing, during this same period the mean per cent variations in $Q_{O_2}$ values for different roentgen dosages, a tendency for a
reciprocal relationship is noted. On the third day however, the variation from the control with larger roentgen dosages is greater. An increase in the roentgen dosages on erythrocytes are known to cause increased hemolysis (Levin and Piffault, 1934; Halberstaedter and Goldhaber, 1943; Frankenthal and Back, 1944). This is not necessarily confined to the time of exposure (Holthusen, 1922; Ting and Zirkle, 1940). Similarly, in these studies, the red cell counts (Figure 7) appear to show a greater decrease (-32%) with 30,000 r. than with lower dosages (-17%). It is suggested that there is an initial, stimulatory metabolic effect in the individual erythrocyte which increases with time and dosage to account for the greater variation in oxygen consumption as compared with the controls. However, it is to be noted that if the results for 15,000 r. are plotted with other roentgen dosages in terms of the per cent variation in oxygen consumption from the controls as a function of time (Figure 9), it appears that the greatest effect is found immediately after x-irradiation. This is followed after overnight storage by a decline in the per cent variation to that exhibited by the 10,000 r., 20,000 r., and 30,000 r. dosages at the same period of time. This suggests that the oxygen consumption from other dosages may possibly give a great stimulatory effect immediately after x-irradiation and then decline after overnight storage,
to be followed by an increase with time and presumably a decrease as dissolution of the cell occurs.

Although there is a decrease in the oxidative metabolism of certain rat tissues subjected to various steroid hormones (Hayano, Schiller, and Dorfman, 1950), it appears that rat erythrocytes in vivo require adrenal cortical hormone for the normal maintenance of their respiratory metabolism which is not increased above a maintenance value by an excess of the hormone (Gonzalez and Angerer, 1947). This lack of effect from excess hormone was demonstrated on adrenalectomized rats and whether this is substantiated in normal rats receiving excess adrenal cortical hormone must await further study.

In view of the changes observed in the oxygen consumption after x-irradiation, it appears that ACE may inhibit, or even reverse the stimulatory effect occurring immediately after exposure. That this action does not persist is evidenced by the return of the oxygen consumption of the ACE-pretreated, x-irradiated red-cell pools to or above, the level of the non-treated x-irradiated red-cell pools after overnight storage (Figure 9). The change in oxygen consumption at 21 hours after x-irradiation may be interpreted as due to the inactivation or utilization of the ACE that was present on, or within the cell, thus permitting the
processes which occur from x-irradiation to proceed as in the non-treated, x-irradiated red-cell pools. The nature of such processes is not known at the present time. However, it is thought that steroid hormones inhibit dehydrogenase activity (Gordon, Bentinck, and Eisenberg, 1951) and, also, that x-irradiations cause metabolic disturbances through enzyme inactivation (Barron, 1949). Thus, it is unlikely that a satisfactory explanation of the results obtained here can be offered on the basis of a single enzyme effect. Since it has been shown that adrenal cortical extract may influence the permeability of red cells to glycerol (Netsky and Jacobs, 1941), it is possible that the action of ACE upon x-irradiation may be through permeability regulation, but whether such action is mediated by the hormone directly on the membrane, or indirectly by way of the respiratory metabolism, is not indicated in these studies.

Stimulatory effects on cellular oxygen consumption due to x-irradiation has been observed on skeletal muscle (Fern and Latchford, 1932), frog skin (Williams and Sheard, 1932), and fowl erythrocytes (Frankenthal and Back, 1944). Although these cells are nucleated, if one is to accept the nucleus as not entirely responsible for metabolic activity (Hunter, 1941; Back and Frankenthal, 1947) and that x-irradiation has its primary effect on
SUMMARY

The respiratory metabolism of rat (non-nucleated) erythrocytes resuspended in equal volumes of Krebs-phosphate-Ringer solution, was studied by the Warburg technique. The studies reported and the conclusions obtained may be grouped and summarized as follows:

1. Aging (storage) on oxygen consumption. When pooled red-cells from "normal" rats are permitted to age in vitro as a result of being stored at temperatures between 2° and 8°C., there is a progressive decrement in their oxygen consumption. This is illustrated when oxygen consumption, either as $Q_{O_2}$ values or as ccm. per billion red cells, is plotted as a function of time in days. These experiments were generally terminated after the sixth day of isolation.

2. Exposures to various dosages of x-irradiations.
   a). Three, two, and eight different batches of pooled (32 to 43 rats per pool, 2 to 3 ml. blood per rat) red-cell suspensions were irradiated with 10,000, 20,000, and 30,000 roentgens, respectively. When oxygen consumption per billion red-cells is plotted against time in days (3 to 4 days) an increase in respiration is observed as compared with the paired controls, for all roentgen doses
suspensions show that while the former give a -8% decrease at the end of the first hour, the latter give a +38% increase. This suggested that ACE, under the experimental conditions involved, protects (antagonizes) the metabolism of the red cell from the stimulatory effect invoked by x-irradiation. After a 21 hour interval the respiration of both pools become congruous. It is desirous to study more red-cell pools relative to this point.

4. Red cells from various clinical patients. The oxygen consumption (\(Q_{O_2}\)) for red-cell suspensions from hypothyroid, iodine-treated hyperthyroid, and hyperthyroid patients show respectively a -15% (\(P<0.20\)), +23% (\(P<0.50\)), and +92% (\(P<0.04\)) variation in \(Q_{O_2}\) values when compared to the values for red cells of euthyroid (control) patients. All other comparisons between the various mean \(Q_{O_2}\) values for the different thyroid groups are not significant except that for hypothyroid and hyperthyroid patients (\(P<0.01\)).
Statistical evaluation of the data for Figure 10 which relates the oxygen consumption of erythrocytes as a function of plasma protein-bound iodine.*

Simultaneous measurements of these two variables were made on 26 individuals. The data is given on page 64. Calling $x$ the protein bound iodine measurement and $y$ the $O_2$ consumption of erythrocytes the data is to be fitted by a curve of the form $y = a e^{bx}$, where $a, b$ are constants to be determined and $e$ is the base of the natural logarithms. This problem is done by fitting a straight line to the logarithms of the $y$'s, i.e., $z = \log y = \log a + bx$.

Partial steps in the computations are given in the table, (page 64), the result giving $z = 0.1395x + 2.0959$. The standard error of $z$ about this regression line is $s_e = 0.343$. Translating this line back into the exponential curve gives $y = 8.133e^{0.1395x}$. The standard error of $y$ about this curve is $s_e = 6.41$.

*I wish to thank Dr. R. D. Whitney, Statistical Laboratory, The Ohio State University, for his council and aid in fitting a curve to these data.*
### Table of Data and Computations

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<td>(x,z) Covariance</td>
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*E(y)* is the value of y computed from the exponential curve.
BIBLIOGRAPHY

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Ponder, Eric, 1949. The rate of loss of potassium from human red cells in systems to which lysins have not been added. J. Gen. Physiol., Vol. 32: 461.


