HAYHURST

Gaseous Exchanges in
Isolated Muscle and the
Perfection of an
Apparatus for
Studying Same

Graduate School
A. M.
1 9 0 5

GASEOUS EXCHANGES IN ISOLATED MUSCLE AND THE PERFECTION OF AN APPARATUS FOR STUDYING SAME

BY

EMERY ROE HAYHURST, A. B., '03

―――――

THESIS FOR THE DEGREE OF MASTER OF ARTS
IN PHYSIOLOGY

IN THE

GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

PRESENTED JUNE, 1905

UNIVERSITY OF ILLINOIS

June 1 190 5

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Emery Roe Hayhurst, A.B.

ENTITLED *Gaseous exchanges in isolated Muscle*

and the perfection of an apparatus for study-
ing the same

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF *Master of Arts*

Geo. T. Kemp

HEAD OF DEPARTMENT OF *Physiology*

CONTENTS .

GASEOUS EXCHANGES IN ISOLATED MUSCLE
AND THE
PERFECTION OF AN APPARATUS FOR STUDYING SAME

This research was undertaken primarily to determine the influence of the nervous system on oxidation in muscle, and to throw more light upon the mooted question whether trophic nerves exist apart from motor nerves. The plan proposed was to study the CO_2-yield from isolated muscles, directly comparing normal muscles with the homologous muscles of the same animal, which had been curarized to throw the motor nerves out of function. Furthermore, to make these comparisons upon both warm-blooded and cold-blooded animals. This involved the wider question of the true character of respiration in _normal_ isolated muscle, and the means of accurately following gaseous exchanges in them.

The work of preceding observers along this line has been strikingly discordant; due to the various conditions, more or less abnormal or injurious, to which the muscles have been subjected to obtain their gases, and the methods employed in estimating these gases.

CONDITIONS -- The conditions which we regard as necessary to be met in order to study the CO_2-yield from an isolated muscle may be briefly summed up as follows :

1. A collection of the CO_2 beginning as soon after excision as possible.

2. A continuous collection thereafter as fast as evolved from the surface of the muscle; or, if possible, from its very seat of origin within the tissue. This is to prevent retention and ab-

sorption of the CO_2 by the substances and juices of the tissue.

3. An immediate removal of the CO_2 from the neighborhood of
the muscle to^forestall any change in the constant yield which might be due
to an increased CO_2 partial pressure in the gaseous medium sur-
rounding the muscle.

4. A means of isolating sharply the CO_2 yielded in a certain
period.

5. An opportunity for obtaining the yields of several consecu-
tive periods from the same muscle without, in any way, blocking
the constant removal of the gases.

6. A means of rapid estimation of the gases obtained in order
to save the experimenter time and labor, and to learn the results
of a certain manipulation in time to decide upon the next.

7. A method of estimating with accuracy as little as 1/100
c.c. of CO_2.

8. Preservation of the tissues under moist conditions for
hours after excision.

9. An opportunity for varying the conditions of experimenta-
tion (as to temperature, pressure,etc.).

10. As simple and inexpensive a method and apparatus as the
various conditions imposed will permit.

HISTORICAL -- The methods of studying and estimating the gas-
eous exchanges in isolated muscle have varied considerably since
Liebig[1] began his experiments in 1850. They may be divided into
gravimetric, gasometric, and volumetric. The inexpediency of the
first two as compared with the third has now become generally ac-
knowledged (especially in their application to physiological
problems), so a discussion of them will not be undertaken. In
the volumetric method, changes in the temperature of the gases

evolved have no influence on the accuracy of their determination.

The trend of opinion as expressed in the latest publications, especially those from England, seems to favor the work and results of Fletcher[2] (who used the volumetric method), as the most convincing. After reviewing the classic experiments of L. Herman[3], Matteuci[4], Valentin[5], and Tissot[6], all of whom found an apparent increased yield of CO_2 in contracting muscle, Schafer[7] goes on to say: "Recently the question has been again investigated by Fletcher, who employed a titration method for the estimation of the CO_2, and made use of the extremely accurate apparatus devised by Blackman[8] for estimating the gaseous exchanges in plants. Fletcher, both with skeletal and with cardiac muscle (tortoise), was unable to obtain ~~only~~ *even* the smallest possible difference of CO_2 output between rest and contraction, and he comes to the conclusion that the contrary results obtained by Hermann and others are due to the prolonged stimulation inducing the commencement of rigor mortis, a condition which is attended by a considerable output of CO_2."

A careful comparison of the researches of these various authors led us to the conviction that an improvement in the method employed was the first essential. Proceeding from this practical standpoint, an apparatus has been devised and elaborated, (continuing the work of Stanley[9]) which was itself a modified and simplified Fletcher-Blackman apparatus. This has been perfected to such an extent as to give an error which is so small as to be well within the limit which the physiological problem demands, and, further, removes most if not all of the objections for which the methods and apparatus of previous observers have been criticised. In subjecting this apparatus to

the most rigorous tests for delicacy, many new and interesting facts have been brought out which will be of decided practical importance both in physiology and in chemistry.

The principal of the volumetric method generally employed is well-known as Pettenkofer's.[10] It consists in passing the CO_2 to be determined through a baryta solution of known strength and titrating back the excess baryta with standard HCl, using phenolphthalein as an indicator. The loss thus found in the strength of the baryta represents the amount of CO_2 absorbed. Pettenkofer's original method was to carry on the absorption of respiratory CO_2 in a Pettenkofer's tube and, after the experiment, to allow the precipitated $BaCO_3$ to settle, then decant a portion of the supernatent liquid, and titrate with the acid as quickly as possible in the air. The results were, of course, subject to error from atmospheric CO_2 .

In 1895, Blackman,[8] in seeking to follow the respiratory exchanges in plants, adopted this method and devised an elaborate apparatus which greatly improved its delicacy and suitability for such physiological purposes. By means of glass tubing and rubber connections for transferring the gas, he made a "closed system" throughout, thus excluding atmospheric contaminations. His plan, in brief, was to force a current of air, by means of a hand-bellows, through a potash "tower", where atmospheric CO_2 was removed. The purified air, so obtained, next passed to the receiving chamber containing the specimen experimented upon, where it gathered up whatever CO_2 was evolved and carried it over to an absorption chamber containing the baryta solution. From here, the air-stream passed through an aspirator-bottle to produce a steady

current..   After a proper interval, the excess baryta could be
estimated in the absorption chamber directly, since the HCl and
baryta burettes and the indicator-holder all opened into the cham-
ber.   (The presence of the Ba CO_3 precipitate does not interfere.)[11]
During such a titration, the current of air from the receiver was
switched to a second absorption chamber, thus avoiding any inter-
ruption to the continuity of the experiment.   He used N/20 solu-
tions.   As to the delicacy of the apparatus, he says:--"Errors
of observation of not more than 1% are often obtained in control
titrations.   This corresponds to 1/200 C.C. CO_2-- In experiments
of short duration, 1/50 C.C. CO_2 is found to be sufficient for a
trustworthy estimation from which definite conclusions may be
drawn."

In 1898, Fletcher[2] adapted the apparatus for his well known
experiments upon "Survival Respiration" in isolated muscle.   He
introduced several modifications in the apparatus to promote still
farther rapidity of manipulation and accuracy.   Among his inno-
vations were "automatic zero" burettes to do away with the reading
of the zero point when titrating, larger stock bottles for the
standard solutions, and the abolishing of India-rubber joints
controlled by clips, using instead three-way cocks.   He also
made an improvement in the siphon tube of the aspirator-bottle
whereby a greater constancy in the rate of flow of the air cur-
rents was secured.   He makes the following claim for the delicacy
of CO_2 absorption by the apparatus: "In testing the completeness
with which the CO_2 is absorbed as the air current bubbles from the
dilated end of the delivery tube through the baryta solution, I
have found that at the rate of flow used in my experiments (from

100--120 c.c. per hour) and when the percentage of the CO_2 in the air was not more than 3%, the absorption of the CO_2 by the baryta solution was very complete, the amount of CO_2 escaping being always less than 1/200 of that absorbed."[13]

In 1902, Stanley, after experimenting with Hempel's and Lunge's apparatus and several other forms, finally adopted Fletcher's method, but with several important improvements. His work resulted in a great simplification of the apparatus and at the same time adapted it for placing the muscle under better conditions for obtaining its CO_2. He used a weaker solution of baryta (N/40) thereby reducing the importance of burette reading errors. Finding that rubber tubing and rubber stoppers yielded uncertain amounts of CO_2, depending upon the temperature, he set up an apparatus in which the air current came in contact only with glass or mercury. To do this he made use of mercury seal joints, the particular form of which is described as the "cup and bell" joint later on in this paper. By this style of joint, he also overcame another great objection to the Fletcher-Blackman apparatus, that of too great rigidity. He further modified the apparatus by dispensing with the hand-bellows and aspirator-bottles, substituting for them an ordinary Bunsen pump attached to the water faucet. This caused the air current to be sucked through the apparatus and to create a negative pressure, thereby overcoming his (and our) objection to Fletcher's apparatus and results, viz: "There is evidence to show that, in Fletcher's experiments, the CO_2 of contraction was simply absorbed by the alkaline fluids of the muscle substance, because there was no negative pressure to draw it out as it was produced. Experiments of Hermann seemed

to indicate this to be true, etc."[15] The Bunsen pump allowed of a greatly increased rate of current while it required practically none of the operator's attention, as was the case with the bellows. The importance of a perfectly steady rate of current[16](so many c.c. per hour) also seemed uncalled for, the main object of each experiment being to obtain all the CO_2 as fast as evolved and transfer same at once to the absorption chamber.   Consequently Stanley eliminated the resistance bottles altogether. Fletcher had used these to keep the current steady when not passing through the baryta solution.   In our own experiments described later on there seems to be some evidence that the tissues handled by Fletcher were too closely confined.   He made use of muscle chambers so small as to be justwithin the limit of practicability; obviously, as he says:- "Firstly in order that the period may be as short as possible which must elapse between the final closing of the chamber and the first estimation which shall fairly represent the CO_2 production, and secondly in order to secure prompt representation in the absorbing baryta solution of any change in the production rate."   However there is some question whether the slow rate of current used, though it may have been fast enough to remove the muscle gases which were evolved before they became detrimental, still furnished the closely confined tissue with enough atmospheric oxygen to satisfy its normal demand.   As evidence in support of this view, we cite Fletcher's results obtained in 1902 wherein he found that--"The survival discharge of CO_2 from an excised muscle is increased during periods of contraction in the presence of abundant oxygen (The muscles had been surrounded by an atmosphere of oxygen).[18]"   We are of the opinion that in his former experiments with air alone,[19] pure air was not

supplied to the contracting muscle in sufficient amount. Further-more, we agree with Stanley in the following:- "In Fletcher's experiments the two corresponding muscles of the same frog were not directly compared, but, for instance, the product of CO_2 from a contracting muscle was compared with the 'normal' curve of production, which had been previously secured from resting muscles. The normal curve which he found varied within rather wide limits so that such comparison does not seem to us just."[20] In place of the potash "tower" used by Blackman for freeing the air current of atmospheric CO_2 , Stanley used an ordinary Winkler spiral, or simply small bottles filled with strong potash. These required much less room than the towers and were thoroughly efficient, as positively no CO_2 escaped them at all.

Our own improvements in the apparatus have resulted from a long series of test experiments upon Stanley's. These demonstrated that a more complete method of absorption than either Fletcher or Stanley had used was essential, and that at a rate of current of from five to eight liters per hour ought to be used in order to insure rapid removal of the gases from the muscle chamber. Other improvements have consisted in a steadier current than Stanley's, a better system of exits and burette attachments to the absorption chamber, a quicker and simpler method for connecting up the muscle chamber, etc., etc., as will be brought out in the description of the apparatus and the methods of manipulation following.

Photograph 1.

AB --- Divides apparatus A (on the left) from apparatus B (on the right).

C --- Muscle chamber.

D --- "Goose-neck" tube leading from CO_2 trap below to E.

E --- Inlet tube of muscle chamber containing thermometer.

F --- Outlet tube of muscle chamber, bent backward as an inverted U.

G --- Left steel supporting rod.

GH --- AB-included between these two bands is one complete absorption system (A II).

I --- Large cup and bell at head of each distributing system (HCl and baryta).

J --- Air inlet tube to each supply bottle.

K --- Mercury cup on end of J.

L --- Three-way T cock distributing HCl to apparatus A and B.

M --- Air inlet tube at foot of "$CaCl_2$" jar.   The jar is filled with soda lime.

N --- Winkler spiral containing 50% KOH.

O --- Interposed bulb to check backflow of KOH toward "$CaCl_2$"jar.

P,P etc.---Mercury reservoirs.

R --- Mercury cup at head of HCl distributing system for apparatusA.

S --- Three-way cock distributing HCl to right hand burette on each titration chamber (I,II)

I, II --- Titration chambers for apparatus A with spiral shown below each.

III, IV --- Titration chambers for apparatus B, with spiral shown below each.

The metronome, with wires attached, is faintly shown

above the left hand gasometer.
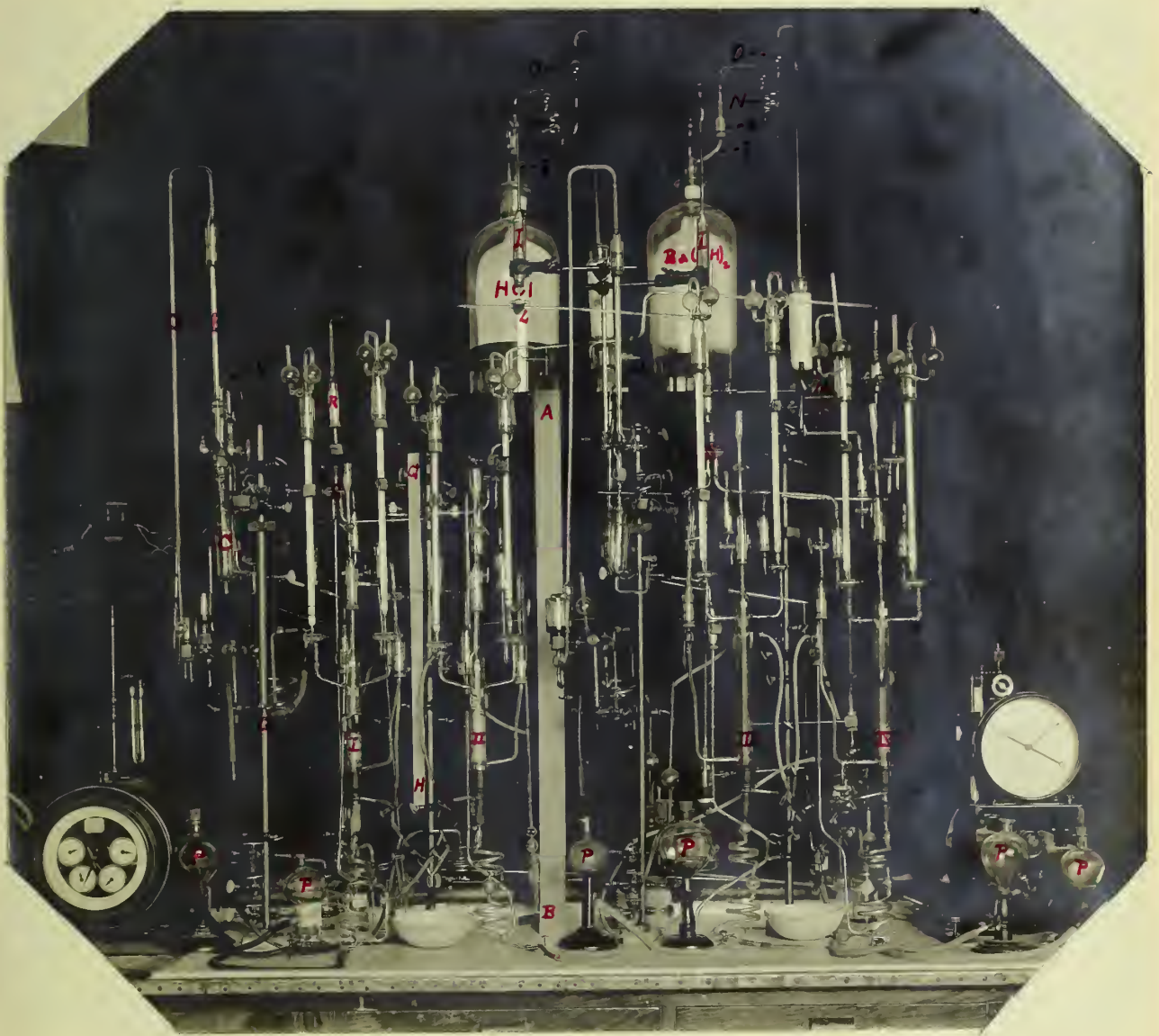
Photograph 1.

Photograph 2.

A --- Regular type of connecting tube having cup (on right end) and bell (on left end).

B --- Elongated bulb at base of absorption spiral.

C --- Air inlet tube to spiral (bringing the air from the muscle chamber).

D --- Mercury inlet tube opening upward into lowest point of bulb B.

E --- Regular bulb on outlet of Winkler spiral.

F --- Additional bulb, elongated and of same diameter as constricted lower portion of H.

G --- Outlet tube of absorption spiral. (In position,this passes through H and ends a little below J in bottom of titration chamber).

H --- "Bottom piece" (supported by clamp to right of chamber),outlet tube on the right.

I --- Air exit tube and entrance for indicator dropper (Y).

J --- Side inlet tube at base of chamber to admit air current for stirring solution during titration.

K --- Mercury cup on top of J (not well shown, as it is behind M and O).

M,M --- Inlet tubes from burettes for standard solutions. Each extends inside the chamber and ends in a dropper.

O,O --- Three-way cock at foot of each burette.

P,P --- Burettes, HCl on the left, baryta on the right.

R,R --- Mercury cups, one on top of each burette.

S,S --- Potash bulbs with bells to fit R,R. (The left one is shown clamped to one side).

T,T --- Mercury cups, receiving standard solutions from distributing system, for each burette.

U --- Air outlet tube passing backward (starts from I just below X).

V --- Mercury cup on top of U for attaching long "goose-neck"tube W (shown on the right).

W --- "Goose-neck" exit tube joining V with a Winkler-Kyll bulb (not shown).

X --- Mercury cup on top of I to support indicator dropper Y.

Y --- Indicator dropper: (1)for indicator supply: (2)one-way cock: (3) bell to fit X having the tube: (4)continuous with "Y",passing through it.

Z --- Mercury cup on top of air inlet tube to spiral. The bell on A fits into this while the cup on A connects with the exit tube from the muscle chamber (not shown).

Photograph 2.

THE APPARATUS.

The apparatus as a whole consists mainly of a combination of standard parts, found individually in any catalogue, simply connected in the proper manner by means of glass tubing, etc., and held in place by suitable clamps. With the exception of the muscle chamber and the titration chamber, which were made to order at small expense, all other parts and connections were made in the laboratory, using an ordinary Bunsen blowpipe. Practically all of the standard parts have remained intact and can be removed from the apparatus at any time and put to other usages. The glass tubing generally used has an inside diameter of 3 m m. With this size tubing, no blocking up by moisture-collection ever happens, as is the case in Fletcher's apparatus with tubing of 1 m.m. bore.

LOCATION.

The apparatus is set up on an ordinary laboratory bench built solidly against the wall. Projecting from the wall, 1.1 meters above the table is a stout shelf 20 c.m. wide, which supports the two large bottles containing the supply of standard baryta and hydrochloric acid. The table is covered with white oil-cloth, which aids materially as a background for determining end reactions. At the front edge of the table it is raised over a strip of wood about 1 c.m. high which prevents mercury from spilling on the floor. The surface of the oil-cloth is advantageous, since mercury,spilled, stops almost where it drops and can be easily gathered into a stationary pool at any place. This may then be scooped over the raised edge of the oil-cloth into a dish by means of a stiff piece

of pasteboard.   The wall back of the table and beneath the shelf is covered with white paper to serve as a background.

The quadrupled absorption system; i.e., having four estimation chambers, as set up for determining the simultaneous gaseous production of two separate muscle masses, occupies a space on the table top 1.2 X .75 meters, and 2 meters high including the supply bottles above.   Four 12-m m. steel rods suffice for the frame work, three of them 1.1 meters and one 1.4 meters long.   The extra long one is to support the upper part of the distributing system.   The sink, with faucets and Bunsen pumps, is on one side; the gasometers immediately on each side.   For convenience the muscle system to the left has been designated A, that to the right, B.   The four absorption chambers have been designated 1, 11,111,and 1V;   or A-1, A-11, B-111,and B-1V.In the accompanying photograph (1), taken with a black background, the broad, white, vertical band AB in the center separates the two systems.   The one set of supply bottles on the shelf above furnishes both systems.

A brief description of only one system is necessary.   This consists of the muscle-chamber C, or receiver, connected by a three-way stop-cock with two estimation chambers, 1 and 11.   All the additional parts shown in photograph 1 are accessory to these, and will be taken up in their proper places in the detailed description following.

JOINTS AND CONNECTIONS.

It is first necessary to describe the type of joint, or method of connection used throughout our apparatus.   In order to overcome rigidity and at the same time to do away with rubber

connections which might have any contact whatever with the gases or solutions handled, all joints have been purposely made loosely fitting and then rendered air tight by mercury seal. Diagram 1 explains the principle. The "cup" is 6.2 c.m. long X 2 c.m. in diameter, and has a No. 3 one-hole rubber stopper inserted at lower end. The "bell" is about same length as the "cup", but only 1.2 c.m. in diameter; this fits loosely over the glass tube, inserted through bottom of "cup", and loosely within the "cup."
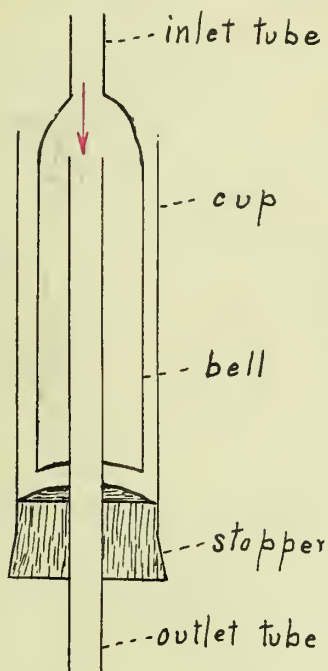
Mercury is then added to about half fill the cup. The joint of course is a vertical one. The same kind of joint is used with both gases and solutions. As the pressure varies within the system, the mercury rises and falls within the cups. The arrow indicates the usual direction of current. The rubber stoppers should be well washed before using, and may be further purified by boiling in acid and in alkali in the usual way.[11]

DIAGRAM 1
natural size

MUSCLE CHAMBER.

But one type of muscle chamber has been used,--a simple tubular compartment 15 c.m. long, over all, and 3 c.m. in diameter. (Shown indistinctly as C in upper left-hand corner of photograph 1.) This easily accommodates all the muscles and muscle-masses ordinarily used in experiments for which the apparatus is intended. About 2.5 c.m. from the top, the chamber has a ground glass joint which divides it into chamber proper and top piece.

(See diagram 2).

From the center of the top piece, hangs a glass hook to suspend the muscle.   Two platinum electrodes are also fused through the top.   The inlet and outlet tubes communicate with the chamber through the top piece on either side of the glass hook.

The air which enters the chamber first passes through a medium sized Winkler absorption spiral con- 50% KOH.   (See diagram 3).   The outlet tube of the spiral ends square against the end of a short glass tube A (a piece of rubber tubing holds the ends together), which passes just through a rubber stopper in the neck of an inverted 60 c.c. wide-mouth bottle, filled about half with 50% KOH. The outlet tube from this begins above the level of the KOH and passes through the stopper of a second inverted bottle containing 1% KOH. The outlet tube from this, also beginning above the level of the KOH, is bent upward and forms a long "goose-neck" D shown in upper left hand part of photograph 1.   To keep the solutions in their proper receptacles during the passage of air, a bulb is interposed between the spiral and the first bottle, also between the two bottles, and in the lower part of the "goose-neck." The "goose-neck" ends in a "bell" which fits within a cup on the upper end of the inlet tube E of the muscle chamber.   The first
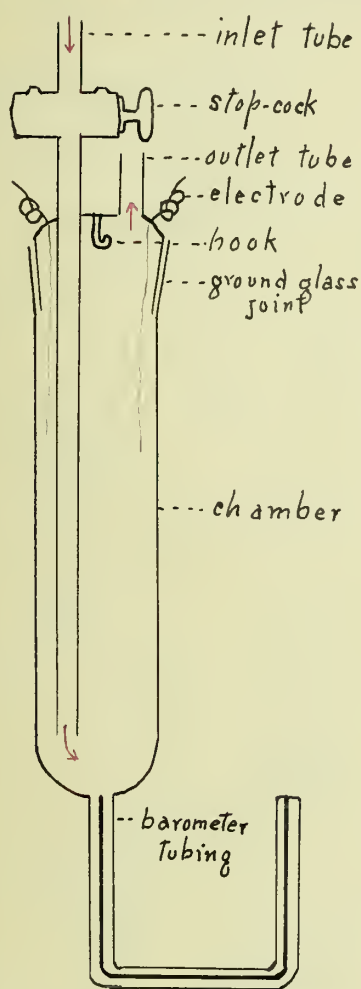
inlet tube
stop-cock
outlet tube
electrode
hook
ground glass joint
chamber
barometer tubing

DIAGRAM 2
½ natural size

part of the inlet tube is long and large enough to accommodate a thermometer within.   A large one-way mercury seal stop-cock is placed in the inlet tube just above the muscle chamber.   (Diagram 2).   The tube passes through the top of the chamber and ends about 1 c.m. from the bottom, when the latter is in position.   The outlet tube begins flush with the top of the chamber and is bent backward as an inverted U, (only partly shown in photograph 1 F). At the further end, this tube is divided by a three-way mercury seal stop-cock, into two branches each of which connects with the spiral of an absorption chamber.   A connecting tube with the "cup and bell" joint already described spans the distance.   (See photograph 2,A). From the bottom of the muscle chamber is a short U tube of barometer tubing.   This may be used for connecting with a vacuum pump, or for inserting an additional electrode by simply pushing a platinum wire through it.   In the latter case, this tube is filled with mercury, and is closed at its outer end by a piece of rubber tubing and a pinch cock.   The method of supporting these parts must be noted. The spiral and two bottles and "goose-neck" are all clamped firmly in their relations to each other, but the whole is capable of being moved up and down by a clamp attachment to the left-hand steel supporting rod.   Thus the whole air purifying apparatus can be disconnected from the muscle chamber by simply raising one clamp.   (Photograph 1, G).   The muscle chamber itself is held in place by a clamp grasping the inlet tube.   The top piece is never removed at all, but the bottom part, or chamber proper, is removed,at the ground joint, to afford access to the chamber. A wire hook,reaching down from the clamp above,retains the bottom part of the chamber in position,in case it should accidentally slip at the ground joint.

THE ABSORPTION APPARATUS.

The absorption apparatus is made in three separate parts.
(See photograph 2). [p.15.] The absorption part proper consists of two
small Winkler spirals having their inlet tubes with "bubblers" re-
moved and then fused together as shown in ~~illustration~~ *photograph*. (By
"bubblers" is meant the constricted end of the inlet tube in the
foot of the spiral which causes the air to bubble through the

50% KOH

A
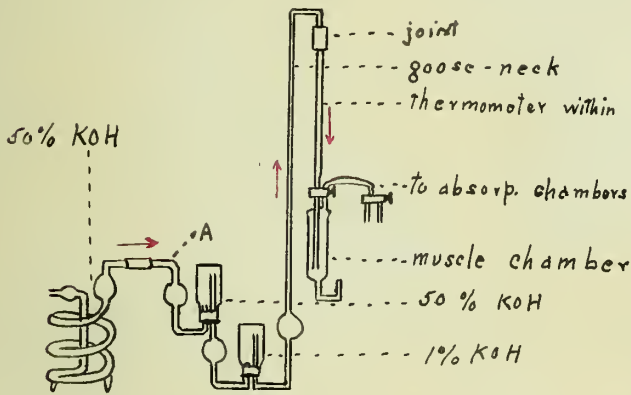
DIAGRAM 3
1/12 natural size
→ See page 19

joint
goose-neck
thermometer within
to absorp. chambers
muscle chamber
50 % KOH
1% KOH

spiral when filled with a
solution). The bottom of
the spiral is expanded into
an oval bulb B (The bulb is
made separately out of a T
joint, one arm of which is
then fused to the spiral) of
about 5-6 c.c. capacity
which receives the air from

the muscle chamber through the tube C. This is bent under the
upper part of the spiral to help support it when filled with mer-
cury.* *(It was found later that this extra bend was really a dis-
advantage, in titrating, while the support it offered was not neces-
sary). The tube D passes vertically downward from the bottom of
the bulb, then is bent sharply to right angles to lie horizontally
on the table. Attached to this is a rubber tube, with a clip,
leading to a mercury reservoir. (Several of these are shown in
photograph 1 supported on pedestals PP). The upper spiral ends
above with its own bulb E to which is fused another bulb F somewhat
drawn out and bearing the glass tube G. When the parts are put
together, this tube passes up through the bottom-piece H fitting

very loosely. (See diagram 4). The bottom-piece (see photograph 2, <u>H</u>) is T shaped. The constricted part, I, rests upon the upper bulb of the spiral. A short piece of rubber tubing, stretched over both, holds them together. The expanded upper arm H slides loosely into the open bottom of the titration chamber. A piece of heavy pure rubber tubing about 25 m.m. bore is stretched over the bottom of the titration chamber and the projecting end wired tightly around the "neck" of the bottom piece. Mercury is then run in to cover the exposed rubber, thus making a mercury seal joint. The side-arm M is the outlet for ~~solutions~~ emptying. A rubber tube and clip N connects it with one arm of a glass T-joint O, the stem P of which ends with another rubber tube and clip, while the arm R joins with

rubber connection --
wire --
rubber connection --
bottom of titration chamber
mercury
--H   .M
--mercury
--I
--F
--E
from spiral
side inlet tube
.N   .O   .R
--P   --S   --X
solution outlet
to mercury reservoir--
--V
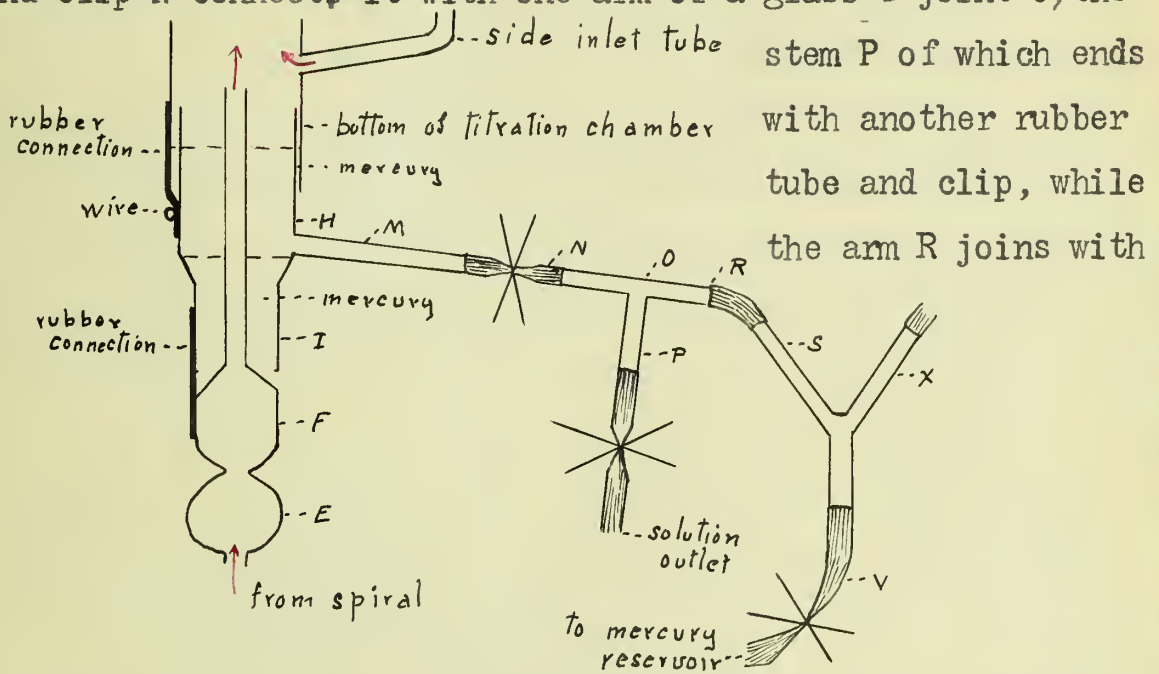
one arm of a Y-tube S. DIAGRAM 4 The stem of the lat- ½ natural size
ter connects by the rubber tube (with clip) with a mercury reservoir. The other arm, ~~of the Y, i.e.~~ X, joins the outlet tube of the neighboring apparatus.
by
The <u>titration chamber</u> is 2.5 X 22.8 c.m., open at the bottom, ~~and~~ narrowed to a 9 m.m. tube at ~~the~~ top I, and extending 15 c.m. high- er. (Photograph 2, large left hand figure). About 5 c.m. from

the bottom is a side tube, J, for bubbling air through a solution dur-
ing titration.   This tube is bent upward and ends with a cup, K,
about even with the top of the chamber.   About 5 c.m. from the top
of the chamber are two more side tubes, M and M.   These, which are
for the HCl and baryta respectively, are fused <u>through</u> the wall of
the chamber.   About 1 c.m. from the inside wall of the chamber,
each is bent downward for about 1.5 c.m., thus making a "dropper."
The inside diameter of the latter is 2 m.m. at the open end.   On
the outside, each is fused with one arm of a three-way stop-cock, O,
bearing a burette, P above.   The burettes, used, are 25 c.c. with
blue enameled strip on white background, known as Schellbach's.[21]
The top of each burette has a cup, R, fitted over it, which supports a
simple "homemade" double potash-bulb S for excluding CO_2 .   The
other arm of the three-way stop-cock connecting with the burette, is
bent in the form of a U, and ends in a cup, T, for connection with the
distributing system which conveys the solutions from the proper sup-
ply bottles above.   The tube I, at the top of the chamber is furnished
with a side tube, U——the air current outlet from the chamber.   This
tube is supplied with a one-way stop-cock and ends in the cup, V.
The long "goose-neck" tube, W, with bells at each end, fits the cup
and serves to conduct the air current onward to a Winkler-Kyll ap-
paratus filled with strong potash.   This is to prevent atmospheric
CO_2 from entering the titration chamber in case the air current is
ever reversed in direction.   The outlet from the Winkler-Kyll appa-
ratus is connected by a rubber tube with the Air-Control System,
which will be described later on.   The tube I ends with the cup X
which is for the insertion of the indicator dropper Y.   This con-
sists of a bell, 1, fused to one arm of a one-way stop-cock 2.   The

other arm of the cock is fused to the special type of bell 3 having the tube 4 passing through it.   This is somewhat constricted at the lower end to form a dropper.   To put the indicator dropper in position, the lower tube 4 is simply slipped down into the tube 1, which is of larger caliber, until the bell 3 fits into the cup X.   The lower end of the dropper should be of such length as to come about even with the baryta and HCl droppers, but should be in the middle between them.

The absorption titration system is entirely supported by two clamps, one grasping the barrel of the titration chamber just beneath the burette inlet tubes, and the other supporting the cup, Z, on the top of the air inlet tube to the spiral.   (Photograph 2). The rubber connections between the spiral, bottom-piece, and chamber also serve to support the spiral.   Thus the spiral hangs suspended from above and only barely touches the table.   This gives plenty of latitude for free play which is of advantage when cleaning up the table top, etc.   As an extra precaution, it is also well to clamp each burette to the nearest support, using ordinary burette clamps, for fear that an accidental knock may break their connections with the chamber.   (They are all shown clamped in position in photograph 1).

CO_2 TRAPS.

The CO_2 trap, used for purifying the air which enters the side tube J (photograph 2) consists of a 200 c/c. plain salt mouth bottle three-fourths filled with 50% KOH and fitted with a two-holed rubber stopper;   the inlet tube, having a bulb above, passes nearly to the bottom of the bottle where it is turned slightly to right angles and constricted.   The outlet tube is S-shaped, the

further end of which enters an inverted 60 c.c. bottle similar to those described with the muscle chamber and containing 50% KOH. From this small bottle, a "goose-neck" tube with a bulb near the bottle, passes upward where it is supplied with a one-way cock and ends in a bell. This later fits into the cup K on the side tube J. The larger bottle of this CO_2 trap stands on the table. The whole trap is an unity in itself and can be lifted off or joined into the system in a moment's time.---In fact, the "cup and bell" joints make the dismanteling or assembling of the whole apparatus a very simple affair, thus greatly facilitating repairs and changes. Any part can be quickly "lifted" out of the system and re-placed or repaired, in some cases even during an experiment.

Both of the air inlet systems, the one to the muscle chamber and the one to the side tube J just described, have a rubber tube on the further side of the CO_2 traps, the further, or distal end of which is within easy reach of the operator. The atmospheric air first enters this tube. Any CO_2 which may be added to it either by the rubber tubing or by the breath of the operator is quickly absorbed by the strong potash beyond. Each tube is com-pressed by a screw pinch-cock. By a delicate adjustment of this pinch-cock, the air current can be made to flow in a perfectly steady stream. This simple arrangement was the most convenient found.

The absorption titration system is multiplied by four to complete the double experiment apparatus as set up (photograph 1); i.e., each muscle chamber is connected with two absorption systems. In photograph 1 that part of the apparatus included between the central white band AB and the shorter band GH includes one absorp-tion system complete. (The taller chambers shown in the right of

the photograph were the first form made and are not as convenient
as the shorter ones shown on the left, and in photograph 2).

AIR CONTROL
SYSTEM.

Apparatus A and B have similar air control systems: A Bunsen
pump is attached to the faucet at the sink, the aspirator of which
connects with a half liter   bottle.   This serves
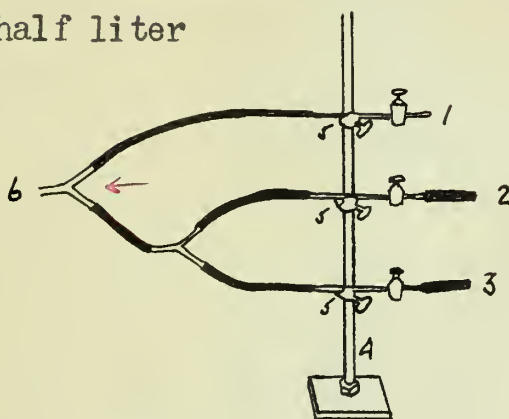
DIAGRAM 5
1/10 - natural size
For explanation of figures, see page 27

to catch the back flow of water whenever the faucet is closed
(amounting to a few drops each time).   The bottle is connected by
a rubber tube to the gasometer, which is in turn connected by a
special arrangement with one of the apparatus, for instance A.

By means of two Y tubes and three one-way cocks, the latter
clamped near the foot of the left steel support,the suction is dis-
tributed to either absorption chamber 1 or 11 of A,or the negative
pressure may be reduced at will,even to zero, by opening the cock 1
(see diagram 5).   This gives the path of least resistance from the
atmospheric air to the aspirator.   With the water flowing,all suc-
tion in apparatus A can be stopped at once by opening 1. With 1
closed, the suction may be thrown into either or both absorption
chambers, by means of the cocks 2 and 3.   The position of these

cocks is within eash reach of the operator.

       #1--Opens to air of room

         2--Connects with outlet tube of Winkler-Kyll potash bulb for first absorption chamber (1).

         3--Connects similarly with second absorption chamber (11).

         4--Foot of steel supporting rod.

         5--Clamps which hold stop-cocks in position.

         6--Connection with gasometer and thence to pump.

On account of the failure of the water pressure occasionally at the height we were working (third floor), a reservoir was placed in the attic about 8 meters above. This was filled through a ball and socket valve which permitted the water to enter only as the level of the water in the reservoir fell. Hence in addition to a reserve water head, this tank furnished a very constant pressure. The water was siphoned out of it through a 1 c.m. iron pipe to the floor below where it was controlled by a screw valve having the Bunsen aspirator pump attached below. As some of the ordinary screw valves proved to be defective, it was found advantageous to attach spigot valves, with quadrant and pointer[22] , which could be opened to the same point each time and insure a very steady current.

REAGENT
BOTTLES.

The Reagent supply bottles, one for HCl and one for baryta, each of 8 liters capacity, are placed on the shelf before mentioned. Two tubes pass through the paraffined cork or special ground glass stopper in the top of each. One of these, for siphoning out the reagent, reaches nearly to the bottom of the

bottle, while above the cork it is bent forward and downward, ending in a bell of some 10 c.m. length about the middle of the height of the bottle (photograph 1, I). This bell fits into the cup at the head of the distributing system. The other tube J permits the entrance of air into each bottle to replace the bulk of the solution as used. It begins flush with the inside of the cork and ends a few c.m. above the cork in the cup K. The CO_2 trap attached to this consists of a small Winkler spiral filled with 50% KOH, and an ordinary $CaCl_2$ jar, filled with soda-lime. The air enters the bottom of the jar at M passes through the soda-lime, thence to the spiral N, the outlet tube of which ends in a bell fitting into the cup K. On account of a back flow in the spiral, occasionally, depending on the expansion of air from temperature, the inlet tube is also protected by a good sized bulb, O.

DISTRIBUTING SYSTEM.

The distributing system for the standard solutions can be traced out in the photograph. It is the same for each reagent. Beginning in the cup I in front of each bottle, a three-way T stop-cock, L, permits of a continuous flow to left and right to supply apparatus A and B respectively. A cup and bell joint R then connects with a second dividing system controlled by the ordinary three-way cock S. Each branch of this ends in a bell which is connected with the proper burette.

An inventory of the complete apparatus in duplicate, as shown in photograph 1, is here given. Glass tubing, stoppers, etc., are omitted. The first list (of standard parts) has the catalogue numbers given in some instances. E. & A. = Eimer and Amend; B. & L. = Bausch and Lomb.

Bottles: 60 c.c., salt mouth----------------------------8

    120 c.c., " " ----------------------------4

     8 litre, tincture ------------------------2

Bunsen aspirator pumps ------------------------2

Burettes: Schellbach's,3-way cock,25 c.c.,E.& A.8658 - 8

Calcium Chloride Jars,-------------------B.& L.13248- 2

Clamps: muffes, -------------------E.& A 5966a-50

   iron, ------------------- " 5965 - 6

   burette, -------------------B.& L.13300-28

Gasometers-- 2

Induction Coil and Cells--------------------------- 1

Metronome--- 1

Pinch-cocks: screw,--------------------B.& L.13324- 4

    " ------------------- " 13326- 4

   spring,-------------------E.& A. 5970-14

Spigots: brass,-------------------------E.& A. 8136- 2

Spirals: Winkler, height 20 c.m.------------------- 2

   " " 13 " -------------------10

   Winkler-Kyll,------------------B.& L.16160- 4

Steel Rods: to accompany muffe-clamps, 15 c.m.------- 8

Stop-cocks: one-way plain 2 m.m. bore---------------16

   three-way plain 2 m.m. " ---------------- 4

   " " T 2 " " ---------------- 2

   one-way Hg seal 3 m.m. "large--------------- 2

   three-way " " 2 m.m. " ---------------- 2

Supporting Rods: steel, 12 " diam., 1.1 m. length-- 3

    " 12 " " 1.4 " " ---- 1

Thermometers: 0° - 100° C------------------------- 2

In addition, the apparatus has the following parts made to order:

DIRECTIONS AND PRECAUTIONS FOR MANIPULATING THE APPARATUS .

In this paragraph will be described in brief the general

Brief Direction for   method of conducting an experiment, after
 Conducting an   which each step, with its precautions,will
  Experiment   be discussed in full. -- The water is first
                        started flowing through the Bunsen pump at
such a rate that the gasometer shows between six and eight litres
of air per hour are being drawn through the apparatus.   A simple
titration is then made in the absorption chamber and the barely
tinted neutral solution let down into the spiral.   If there is no
change in the color of the solution by so doing, it shows the walls
of the chamber and spiral are also neutral.   The mercury is now
raised, pushing the solution on ahead of it out of the spiral, and
the solution is allowed to pass out through the exit tube.   Baryta
is then dropped into the chamber and let down into the spiral.   The
air current is now short circuited by opening the stop-cock 1 (dia-
gram 5).   Thus, in order to stop the air current from flowing
through the apparatus, it is not necessary to disturbe the aspirator
pump.   Next, the muscle mass is excised, hung by a platinum wire
from the hook in the top of the muscle chamber, the bottom of the

chamber placed in position, the inlet and outlet cocks properly turned, and the air current started through the system again. The air first enters the rubber tube attached to the large spiral containing KOH (see diagram 3),then up the "goose-neck" and down where the temperature is taken, and enters the muscle chamber. From here it passes out and enters the bottom of the spiral containing the baryta. The bubbles pass up through this, enter the titration chamber above, whence the air passes out through the exit tube at the top, then down through the Winkler-Kyll bulb below to the air control or distributing system, from which it passes through the gasometer to the pump.

The preparation and preservation of standard solutions, the Detailed Directions.

The Solutions.   indicator, and the mercury have all to be carefully noted.

After considerable experimenting with different strengths of standard solutions, we at last decided that $N/55.5$ was the best suited to our purpose. Blackman and Fletcher used $N/23$, one c.c. of which is about equivalent to $1/2$ c.c. CO_2 (at $0°$ C. and 760 m.m.). Stanley used an $N/40$ solution, one c.c. of which is equivalent to 0.272 c.c. CO_2 . On account of the importance of burette reading errors, it is desirable to use as weak solutions as other conditions will permit. We experimented considerably with $N/100$ solutions, one c.c. of which is equivalent to 0.111 c.c. CO_2 , and found that, although the end reaction in 40 c.c. of solution could be read to one drop (from our burette droppers = .07 c.c.) with phenolphthalein as indicator, still the solution was too weak to insure complete absorption of the CO_2 in an air

stream of eight to ten liters per hour. In making these tests, we utilized the ordinary room atmosphere which contained enough CO_2 to neutralize 3 c.c. of N/55.5 Ba $(OH)_2$ in five minutes, or about ten times the amount a good sized muscle would yield in the same length of time.

First an N/10 Na_2CO_3 solution is prepared.[23] Then N/10 HCl, using a few c.c. of concentrated HCl and about two liters of boiled and cooled distilled water; i.e., free from CO_2 . This is stand-
N/55.5 HCl Solution.

ardized by the Na_2CO_3 solution, using methyl orange as an indicator. From this stock solution, N/55.5 HCl is made by diluting. The diluent is prepared by boiling distilled water in 10 liter flasks (to expel all traces of CO_2, fitted with inverted condensers. The outlet of each condenser is fitted with a Varrentrapp bulb filled with 50% KOH. During ebullition the condensed steam drops back into the flask while the expanded air bubbles out through the trap. In the subsequent cooling, the KOH frees the incoming air of its CO_2 .--- In the meantime the 8 liter supply bottle is carefully cleaned and dried (See Appendix, Notes 1 and 2). As soon as the CO_2 -free water has cooled down to a "luke warm" temperature, the condenser is removed from the flask and the 8 liter bottle is about 4/5 filled with the water. The transfer is made as quickly as possible (See Appendix, Note 3). Next about 1500 c.c. of the N/10 HCl is added, which nearly fills the bottle. This is then accurately standardized to N/55.5 by titrating (in proper proportion) against N/10 Na_2CO_3 with methyl orange. The bottle is carefully stoppered and the solution is used later to standardize the baryta.

N/55.5 Ba(OH)$_2$ Solution.---

A stock solution of Ba(OH)$_2$ is prepared by saturating a liter of recently boiled distilled water with 60 grams of absolutely pure alkali-free Barium Hydrate[24] . (See Appendix, Note 4). An 8 liter supply bottle is cleaned and dried (See Appendix, Notes 1, 2, and 5). About 7 liters of "luke warm" CO_2-free water is now quickly poured from the flask into the bottle (See Appendix, Note 3). Then 500-600 c.c. of the saturated baryta solution (which is about N/5) is decanted directly into the bottle, the latter well shaken, and, when at room temperature, titration against N/55.5 HCl begun, using phenolphthalein. As samples of either acid or alkali are needed, they are quickly removed from the respective bottles by means of 100 c.c. bulb pipettes and dropped into the burettes. The burettes (of 50 c.c. capacity) have been previously calibrated. The titration is performed as quickly as possible in a 100 c.c. Florence flask, always adding the baryta first. (Any $BaCO_3$ precipitate formed in the interval by contact with the air is not acted upon by the acid in a careful titration to neutralization.) The titrations are continued until the baryta is apparently of the same strength as the acid. Should the Ba(OH)$_2$ as prepared show any cloudiness or sediment, it must be filtered into another 8 liter bottle, previously cleaned and dried as explained. (For the technic of filtering, see Appendix, Note 6).

The corks, or special glass stoppers, containing inlet and outlet tubes, as described under the apparatus, are now used to close both the bottles of standard solutions and are carefully paraffined in. The bottles are placed in their proper positions

on the shelf and connected up with their respective distributing systems and CO_2 traps. (For special methods of preserving the baryta solution, see Appendix, Note 7).

For special methods of detecting the presence of CO_2 in standard solutions, see Appendix, Note 8).

1% Phenolphthalein -- The purity of the yellow crystalline powder generally bought for making this indicator is of importance, and the delicacy of any sample should always be tested.[24] (See Appendix, Note 9). Although most books direct to dissolve 1 gm. of phenolphthalein in 100 c.c. of 50% alcohol, a better and clearer solution can be more quickly made by dissolving 1 gm. in 53 c.c. of 95% alcohol and then adding 47 c.c. of distilled water. The indicator must then be neutralized. We found that it required from 2-4 c.c. of N/100 $Ba(OH)_2$ (NaOH or KOH must not be used) to produce a faint redness in our solutions. When this point is reached, a drop of N/100 acid is used to restore neutrality.

(For the effect of CO_2 in standard solutions titrated with phenolphthalein, see Appendix, Note 10).

Mercury.--Our mercury is stored and at the same time purified in two large heavy Woulff bottles. New mercury is purified by placing in porcelain evaporating dishes and covering to the depth of a centimeter or so with strong HNO_3 . This is left for several days. The acid is then removed, the crystals of $HgNO_3$ picked off, and the clean mercury below is dried. Both Woulff bottles are then partly filled with the pure mercury. Next, a siphon, such as is ordinarily used for acid supply bottles, is fitted through one hole of each of the Woulff bottles, and pushed to

the bottom of the bottle (below the surface of the mercury).   The
mercury is then covered to the depth of 2 - 3 centimeters with
conc. H_2SO_4 and a liberal amount of powered Hg_2SO_4 added.   A fun-
nel, with stem drawn out to a fine point and turned to an oblique
angle at the end, is fitted through the other hole of the bottle.
Mercury to be cleaned is first dried of any moisture (so as not to
weaken the acid) and poured into the funnel of the first bottle.
The fine hole shoots a stream of mercury against the wall of the
bottle where it is broken up into little drops and falls into the
acid.   This cleanses it from any organic matter.   If any of the
mercury has become amalgamated by coming in contact with foreign
metals during its use at the apparatus, the Hg_2SO_4 reduces the
amalgam to mercury and the corresponding sulphate of the metal.
The mercury so released joins the pure mercury beneath the acid
while the metallic sulphate remains above in solution.   As a
further safe-guard, the mercury is passed through a similar process
in the second bottle.   The mercury for use is therefore only tak-
en from the second bottle.   It should come out of the siphon per-
fectly dry.   It will be found to be neutral in reaction by pouring
a little distilled water on the surface of a sample and adding a
trace of methyl orange.-- The bottles stand on a strong box and a
large, porcelain dish is placed beneath each siphon to catch any
accidental leaks.

KOH Solution -- This is made in two strengths, one 50% for
filling all spirals and bottles intended to entrap atmospheric CO_2,
and 1% to put in the last bottle before the air enters the muscle
chamber, as a means of saturating the air with moisture.   The
strong solution will maintain its efficiency for several months in

the face of almost constant usage.

The Manipulation of the Absorption Apparatus and Connections

After arranging the bottles of standard solutions in place, the next step is to fill the distributing system and burettes.

Burettes

The mercury cups are filled to the proper height with mercury. A CO_2 trap is lifted off the top of a burette, and by means of suction through a rubber tube attached to the top of the burette, the solution is siphoned over from the bottle. By pulling steadily, most of the air bubbles can be removed from the distributing system. Those which remain are in no wise detrimental. After starting the siphon, the flow is controlled by the stop-cocks below. The tops of the burettes should be 3 - 4 centimeters below the bottoms of the bottles, so that they will continue to fill easily when the solutions get low in the bottles. The bottles should not be placed higher, because the pressure in the distributing system then becomes great enough for the solutions to overflow the mercury cups. After filling a burette to the top, it is necessary to fill the tube leading from the three-way cock beneath the burette to the titration chamber. No air bubbles should be allowed to remain in this tube since it conveys the measured solution to the chamber. Air bubbles, expanding with the room temperature and exposed to the negative pressure in the titration chamber, would force out a little of the solution unmeasured. To obviate this, the burette, filled to the top, is turned full force into the chamber, when the pressure of the column of liquid in the burette is great enough to drive all air bubbles down into the chamber.

We now come to the method of getting an even and readable meniscus in the burette. This question seems to have menaced Blackman and Fletcher to some extent as the former claims he could not raise his bottles above the burettes, because the pressure would then raise the surface of the liquid in the burette, while a subsequent drop backward is generally necessary to secure a good meniscus. On account of this, he placed his supply bottle on the table and forced the liquid up into the burette by air pressure, after which he could permit it to drop a few millimeters to produce a proper meniscus. The Schellbach milk-glass back burettes which we used are read by a pointer reflected in the liquid,[21] and a perfect meniscus is essential. To secure this the burette is opened to the distributing system as though to fill, but the three-way cock in the distributing system immediately above is kept closed. It will be noticed that a mercury cup is interposed. The operator grasps the tube of the distributing system and gently moves the bell up and down in the cup. The free play in the system easily permits this. A rise and fall of a full centimeter is thus produced in the surface of the liquid in the burette. This fluctuation in the level of the meniscus is completely under control of the operator, and, when opposite the mark desired, the stop-cock at the bottom of the burette is closed.-- If, by accident, the burette is filled more than a centimeter beyond the point desired, the excess can best be removed by lifting off the CO_2 trap at the top of the burette and sucking out some liquid with a glass tube drawn out to a point.

The cleaning of the burettes is another matter of great importance. About the only source of contamination is a minute

amount of grease carried down from the vaselined stop-cocks in the distributing system.   Much of the difficulty is forestalled by the careful use of vaseline.   All cocks which convey liquids should have barely enough vaseline to keep them from sticking.   When applying the vaseline, care should be taken not to come too near any of the holes.   On the other hand, if there is not enough vaseline, the cocks will invariably leak.   In spite of the most careful precautions, the walls of the burettes will generally show greasiness and a tendency to hold back little drops of the solution after vaselining any of the cocks in the system above. To do away with this, we have made use of a swab, 4 - 5 c.m. long, the core of which is permanently fixed by wrapping a strip of cloth around the end of a copper wire and clamping the end of the wire back to hold the cloth in place.   The wire is again bent to pass backward over the swab.   To use, the swab is partly bent away from its wire handle and wrapped two or three times with a strip of thin tissue paper, 4 - 5 c.m. wide, then the wire bent back tightly in its place to hold the paper in position.   A few drops of ether are then poured on to the swab and the latter inserted into the top of the burette.   The swab should never come in contact with the liquid.   It should be just large enough to fit the inside of the burette easily.   Two or three wipings up and down will usually suffice to remove all of the grease.   Not enough ether should be poured on the swab to be squeezed out and collect on the surface of the liquid in the burette.   A new strip of tissue paper should be used each time.   If much liquid is sticking to the sides of the burette, one or two papers should first be used in absorbing it up;   then a paper soaked with ether inserted to absorb the grease.   It requires only a few seconds to change the

tissue paper on the swab.   Care must be taken not to expose the
tops of the burettes to the atmosphere any longer than possible.

After filling the distributing system and the burettes, it
next becomes necessary to standardize the baryta solution with
great accuracy.   It will be remembered that it was approximately
of the same strength as the acid before putting the bottles in place,
but the titrations were made in the air where the baryta solution
suffered a slight loss by atmospheric CO_2.--   The first portions of
the solutions in the distributing system and the burettes is run off,
without measuring, into the chambers, which have been previously
freed of any atmospheric CO_2 by replacing the air,--a matter of five
minutes with an air current of ten liters per hour.   The waste so-
lutions in the chambers are neutralized and then passed off through
the solution-exit tubes.   The solutions are then titrated in the
chambers in the regular way, adding the baryta first.   The acid is
always taken as the standard, for it is not subject to the changes
(See Appendix, Note 5), which affect the baryta solution.   The ti-
trations are made through all of the chambers in succession, so as
to fill and flush all the burettes and chambers alike, and they
should be continued until the readings for all of the chambers are
the same (correcting, of course, for the burettes).   Usually, with
new solutions, about three titrations in each chamber will be nec-
essary.   In this final standardization, the baryta will always be
found stronger than when titrated in the air.   Whatever the strength
of the baryta solution found, it is expressed in terms of the acid;
i.e., in terms of N/55.5.   For instance, 1 c.c. of baryta may be
equivalent 1.01 c.c. of the acid.   It is accordingly given the lat-
ter value.

Evaporation, or any change in the height of the liquid in the burettes on standing for several days, does not take place to any noticeable extent.   Faulty stop-cocks must, however be guarded against.   (See page 58).

When ready to begin an absorption experiment, both of the Absorption and burettes attached to the absorption chamber, are Titration. filled to about the zero point and the menisci adjusted as above.   The mercury is raised in the chamber to the level of the side tube J (See Photograph 2 and Diagram 4, "side inlet tube").   The air current is started so as to create a negative pressure in the chamber, the air entering through the side tube. Then 24 c.c. of baryta is run into the chamber.   The last drop is cut off sharply so as to leave no drop hanging on the end of the dropper.   (See Appendix , Note 11).   The mercury is now lowered in the chamber and spiral by allowing it to flow back into the mercury reservoir attached to the tube at the bottom of the spiral (See photograph 2, D).   The solution is thus drawn down until it fills the lower bulb of the spiral. The air current is then stopped by opening the cock 1 (Diagram 5).   If satisfied of the perfect neutrality of the chamber and spiral before this operation, we next proceed to prepare the muscle and enclose it in the chamber. (For methods of preparing muscles, see protocols of experiments later on).

The absorption of the CO_2 to be determined is now begun. The air current is again started through the system, the cock connecting the spiral to the muscle chamber is opened, and that to the side tube J of the absorption chamber is closed.   The air, in the shape of pea-sized bubbles, enters the baryta solution in the lower

bulb of the spiral and passes up through the same. The bubbles proceed with short rushes and halts, no matter how steady the current may be which enters the muscle chamber. They are continually broken up and reformed, always in contact with the baryta solution, until they emerge from the surface of the solution, which stands a centimeter or two above the mercury filling the joint at the bottom of the chamber. The speed of the air current is set at eight liters per hour. (At twelve liters, a slight loss of absorption will ensue. The operator soon learns to judge the speed with sufficient accuracy by watching the bubbles in the spiral. The gasometer, however, is always left attached). The great bulk of the CO_2 is caught in the bulb of the spiral in its first contact with the baryta. Fully nine-tenths of the $BaCO_3$ precipitate lodges at once here and in the lower one-third of the spiral. It must be remembered that each air bubble which enters the solution contains a few molecules of CO_2 and the object of the whole absorption scheme is to bring these molecules in contact with the molecules of $Ba(OH)_2$ before the air bubble gets through the solution (a matter of about three seconds). The air bubbles keep the solution well stirred so the solution in the lower bulb is constantly replenished. This lower bulb is a vast improvement on the ordinary Winkler spiral, for it keeps a large supply of baryta solution on the first attacking line.-- No more than 5 c.c. of the baryta solution should be overcome by the CO_2 in one period as complete absorption thereafter becomes uncertain. If much CO_2 is to be absorbed, it is advisable to shunt the current into the other absorption spiral at shorter intervals.

At the end of the period the mercury is raised to about half

fill the lower bulb, so that the air will force its way through and carry over any of the solution which may have gotten on the wrong side of the mercury. The cock at the muscle chamber is now turned to switch the air current into the second absorption chamber, which has been previously filled and made ready. Then the mercury is steadily raised in the first spiral, pushing the solution ahead of it, until the mercury reaches the level of the side tube J. (The solution may or may not be clouded, according to the amount of the CO_2 absorbed during the period. A great advantage of the spiral is that the bulk of the precipitate remains behind lodged on the walls. Thus the danger of hiding the end reaction by the presence of too much precipitate is entirely obviated.) The cock controlling the side tube J is slightly opened so as not to deflect too much of the current from the second spiral. Four drops of phenolphthalein are admitted from the dropper above, the zero point on the acid burette is again noted, and the acid is admitted, going slower as the neutral point is approached. Care must be taken never to overshoot the neutral point with acid, for the $BaCO_3$ precipitate is at once attacked. Before reading the burettes, the solution must be again dropped into the spiral to flush it out, when it will be seen that enough alkali is recovered to about equal two or three more drops of acid. This alkali had remained adhering to the walls in spite of the mercury. After reneutralizing, the mercury is again dropped in the same way, but this time the neutral solution let down very seldom gathers up any more alkali. The mercury is lowered this time until the solution can pass around and up the air inlet tube (coming from the muscle chamber) to gather up any alkali which may have remained on the "wrong side" of the mercury. This operation is facilitated by releasing the negative pressure in this one

absorption chamber. The solution which has gone up the air inlet tube is recovered by re-establishing the negative pressure, and can be hastened by turning the cock at the muscle chamber as though starting the air current through again. The two or three bubbles of air from the muscle chamber which may be caught by so doing may be entirely neglected as that much air will contain no estimable amount of CO_2. The solution is again pushed up into the absorption chamber, its neutrality noted, and the burette readings taken, corrected, of course, according to previous calibration.

The question may now be asked whether the mercury, as it flows back into the reservoir, may not have some of the solution sticking to it in the same manner that it adheres to the walls of the chamber. This, however, is not the case as is easily demonstrated. A solution of baryta may be flushed up and down any number of times in the clean spiral and then titrated as above without showing any loss whatever.

At the end of a titration, to empty the chamber, the negative pressure is released, the clip on the outlet tube is opened (diagram 4, N) and the mercury allowed to flow back into the reservoir. As soon as the bottom level of the solution has entered this tube as far as the exit tube P the mercury is stopped and the clip on the exit tube opened. One or two c.c. of mercury will fall into the dish below, followed by the solution. To hasten the process, the operator may apply positive pressure to the absorption chamber through the side inlet tube J. This is done by blowing into the rubber tube leading to the CO_2 absorption system on that side. His breath is of course freed of its CO_2 by passing through the potash.-- After the solution has passed out, the

mercury is raised, and the chamber is again ready for the baryta
for the next period.   The few drops of neutral solution remain-
ing in the chamber are disregarded as they can in no wise affect
the baryta to be added.   This is a great convenience, as Black-
man observes, for the chambers do not have to be cleaned out and
dried, or even exposed after using.

        This whole process of titration requires but from three to
five minutes.

        The action of two particular stop-cocks must be thoroughly
learned to prevent mishaps;   one connecting the particular ab-
sorption system with the air control system (which see),and the
one on the side inlet-tube J.   By the simple manipulation of
these two cocks, the pressure in the system may be controlled at
will, and may be made negative, atmospheric, or positive.   This
is much simpler than the intricate sequence in the Blackman appa-
ratus.   The only accident which can happen in confusing the se-
quence of action in our stop-cocks is the pulling over of mercury
from a cup.   In this case air will enter the system, but as three
out of the four cups in each system are beyond the absorption
chamber this need cause no apprehension as far as they are con-
cerned.   The fourth cup is on the side inlet tube J and must be
prevented from pulling over.   To do this, one of the cups "beyond"
contains the least mercury, hence is the least resistant, and acts
as the safety valve for the whole system.   Any mercury which is
pulled over from a cup by accident simply passes down to join the
mercury in some cup or other compartment below and can do no harm.

        The $BaCO_3$ precipitate must be prevented from accumulating
on the walls of the spiral.   (See Appendix, Note 12.)

After long use, the walls of the chamber may be found to have accumulated a slight amount of grease. This is brought in by the solutions from the distributing system above. This is easily cleaned by removing the bottom of the chamber and wiping with a filter-paper swab, wet with ether.

Nearly all that need be said of the muscle chamber has been given under the description of the same (see

Manipulation of the

Muscle Chamber page 18'. The CO_2 in the atmospheric air is

generally included in the first period of absorption and then corrected for. The CO_2 content of the air is fairly constant, being about .03 of 1%. Therefore, with the capacity of the muscle chamber at 100 c.c., about .03 c.c. of CO_2 is the necessary correction. As a matter of fact, a number of tests showed the presence of .03 - .05 c.c. CO_2 in a room more or less confined and having one or two free flames burning. This method of correction appeared to us more desirable than Fletcher's plan. With a chamber of 20 c.c. capacity, he waited 10 minutes for the removal of the original atmosphere before he began the absorption. Accordingly, there was a loss of 10 minutes in beginning the experiment. We found the correction so constant and so easy to be made that we preferred to avoid this loss of time. We have, however, the same alternative as Fletcher, and with our faster current, the interval can be set at three minutes, even for a 100 c.c. chamber.

The time required to remove all the gas in the chamber at a certain speed of current was determined by two methods. First the chamber was connected up with an empty absorption chamber, and the air allowed to run for, say, three minutes. It was then switched to a second absorption chamber containing baryta. If

there was no loss in _this_, the CO_2 must have been entirely removed
from the muscle chamber within the first three minutes.   The second method
was to fill the muscle chamber, through the inlet tube, with cigar
smoke, then start up the current.   The time it required to remove
the last traces of smoke was a rough test of the time necessary to
remove a gas.   The shape of the muscle chamber is of importance.
(See Appendix, Note 13).

No solution for moistening the air current should be placed
in the muscle chamber on account of its uncertain influences on CO_2
(See Appendix, Note 10).   The 1% KOH solution, which the air pas-
ses through immediately before entering the muscle chamber, keeps
the muscle very moist.   A little bichloride should be added to it
to prevent bacterial growth.

Because of the negative pressure employed, any leakage in
the apparatus, due to flaws in the glass or improper connections,
     To Prove "No Leakage"   would result in the entrance of air
      in the Apparatus   from without, accompanied by CO_2 .
There are two methods for proving the reliability of the apparatus
in this respect.   The first consists in developing a negative
pressure in the system, then shutting the exit stop-cock, and
watching the mercury cups.   The mercury should remain for hours
at the same level in the cups to which it was first pulled by the
negative pressure.   By this means any communication whatsoever
that may exist between the atmosphere and the closed system will be
discovered.   The second method comprehends the first and is in
reality a control method for demonstrating the trustworthiness of
the machine in action.   All CO_2 is first removed from the whole
system by displacement with pure air, then baryta is run into the

absorption spiral and the current allowed to run for hours.   In addition to defects in the apparatus, this will prove complete absorption of atmospheric CO_2 by the CO_2 traps.   We have conducted such experiments for twenty hours with no loss in the baryta. Faulty stop-cocks in the air system must be guarded against. (See Appendix, Note 14).

Blackman demonstrated the efficiency of his apparatus by

Proof of the Efficiency of CO_2 Transportation and Absorption by the Apparatus

liberating CO_2 from a generator containing lumps of marble, using N/50 - N/1000 HCl, drop by drop.

By this method, he secured a fairly constant supply of CO_2 .   By attaching one absorption chamber to the other, he could tell the efficiency of absorption in the first.   However, this gave him no opportunity of measuring the absolute amount of CO_2 liberated, nor the speed and efficiency of its transportation to the absorption chamber.   By increasing the amount of acid supplied, the CO_2 liberated and absorbed was relatively greater, but great accuracy in the relative amounts was not obtainable by this method.

We determined from the first to test the absolute reliability of the Pettenkofer method as modified by our apparatus.   In other words we determined to liberate a certain definite amount of CO_2 in the muscle chamber or in some other vessel similarly placed, and study the efficiency with which this was transported to, and absorbed by, the baryta solution.   This we soon discovered was a far greater task than we at first anticipated, and lead to a long series of experiments involving the accuracy of production and absorption of the gas.

From the first, the plan was to free the gas from a known

amount of Na_2CO_3 solution, using standard acid. This could be done by connecting three of the absorption chambers in a row, freeing the gas in the first, catching it in the second, and using the third as a check upon the efficiency of absorption in the second. The first chamber would correspond to the muscle chamber. The Na_2CO_3 solution was dropped from a burette with dropper, substituted in place of the phenolphthalein dropper, into a measured amount of acid in the first chamber. By this means the standard acid solution could be used throughout the whole operation. The indicator was at first admitted to the chamber through the baryta burette, but later by an attachment to the exit tube below.

Simple though this may seem upon first sight, we found that we almost invariably caught more CO_2 than was recorded as freed. The difficulty, we learned by experiment, lay in the uncertain CO_2 content of the carbonate solution, whose strength was at first known only in terms of the sodium, and in the amount of CO_2 which would remain dissolved in the acid solution used to free it.

If a carbonate solution contains bicarbonate, the CO_2 of

The Character of an
Na_2CO_3 Solution

the latter is freed by one-half the amount of acid necessary to free the CO_2 from the carbonate. Furthermore, the hydro-
lytic dissociation is so great in a weak solution of Na_2CO_3 (we used N/40, N/90 and N/100) and so affected by exposure, tempera-
ture, etc.,that it is well-nigh impossible to maintain an equilibrium in the CO_2 content of such a

solution long enough to get a series of consecutive experiments.
It is impossible to tell anything about the CO_2 content of a
solution of Na_2CO_3 , made up in the usual way, by an estimation of
the Na.   Even when the solution was made of the pure salt care-
fully fused and every precaution taken to exclude extraneous CO_2 ,
test experiments showed that the solution contained more CO_2 radi-
cles per measured quantity than could be accounted for as previ-
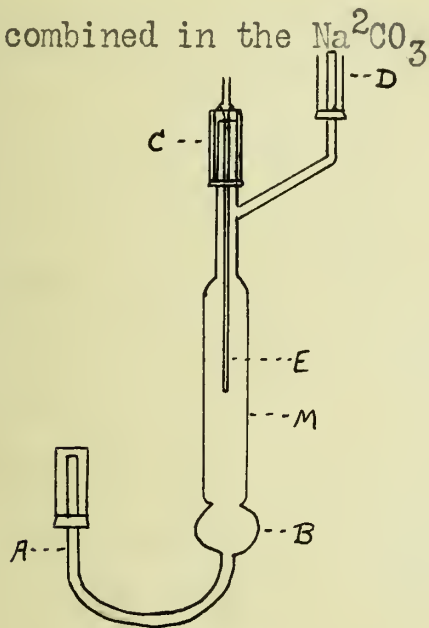ously combined in the Na^2CO_3 .

DIAGRAM 6

1/5 natural size

(A full discussion of the disso-
ciation and equilibrium in a weak
solution of Na_2CO_3 is given by
Herbert N. McCoy, of the Univer-
sity of Chicago, in the American
Chemical Journal, 1903[X]).

On account of the absolute
difference in the end reactions
of phenolphthalein and methyl
orange,[27] the use of the latter
was dispensed with by dropping
the measured quantity of Na_2CO_3

into excess standard acid and then titrating back with baryta.
This gave a fairly clean end reaction with the indicator.   But on
account of the difficulties above mentioned, coherent results were
unobtainable.   An attempt was made to determine the total amount
of CO_2 in the carbonate solution by the gravimetric method, using
Geissler absorption bulbs and freeing the CO_2 in one of the chambers
using the standard acid, but this failed because of the large per
cent of the gas remaining dissolved in the acid.

A special form of chamber for freeing the CO_2 was made (see diagram 6).   A few c.c. of 60% H_2SO_4 was introduced through

The Special CO_2 A until it about one-third filled the bulb B

Liberator   which had a capacity of about 8 c.c. Mercury

cups were now put on A, C, and D.   An N/10 Na_2CO_3 solution was made by dissolving 5.3 Gms. of the powdered crystalized salt [23] in one liter of boiled and cooled water.   A part of this was made N/100 by further dilution with CO_2-free water.   A burette with a one-way cock and dropper was filled with the solution and the top protected by a CO_2 trap.   The dropper E of the burette was introduced into the chamber M.   A CO_2 absorption apparatus was attached to A, and D was connected with the spiral of one of the absorption chambers.   After filling the cups with mercury (thus making the combination system air-tight), the air current was started, entering the CO_2 Liberator at A, bubbling through the strong acid in B, and passing out at D to the absorption chamber. When the whole system had been freed of atmospheric CO_2, baryta was let into the absorption chamber, and a few c.c. of the Na_2CO_3 solution
^-- carefully measured -- allowed to fall on to the surface of the strong acid in the Liberator.   The CO_2 thus freed was carried out through D to be caught and estimated in the absorption chamber beyond.   The strong acid with the air bubbling through it in this manner probably offers the best conditions that can be obtained to prevent reabsorption of the CO_2 by the freeing solution. In fact, after allowing five minutes for the gas to pass over from the Liberator, the current was switched, on one occasion, to another absorption chamber filled with baryta. After thirty minutes, this was titrated with no loss in the strength of the baryta, thus proving that all the CO_2 had been removed from the acid in the Liberator--and

within five minutes. The advantage of the bulb B on the Liberator
is that it prevents any splashing on the walls of the chamber
above. In this way, all of the Na_2CO_3 solution admitted must come
in contact with the acid and there are no wet walls to absorb any
of the CO_2 before it reaches the baryta solution.

The results of six consecutive experiments were strikingly
concordant and are here reproduced:

(The solutions were N/100).

Experiment	Na_2CO_3 Used	Ba(OH) Used	or	CO_2 Freed	CO_2 Caught
1.	27.78 c.c.	2.85 c.c.	"	.31 c.c.	.32 c.c.
2.	2.76	2.82	"	.31-	.31 +
3.	2.81	2.93	"	.32	.33
4.	2.81	2.95	"	.31+	.33
5.	1.39	1.48	"	.15	.16
6.	4.47	4.58	"	.50-	.51+

The "CO_2 Freed" column means the amount of CO_2 which should
have been contained in the corresponding amount of N/100 Na_2CO_3 .
Now the "CO_2 Caught" shows that a trifle more was taken up in
each case than was theoretically freed. As control tests before
and after showed no leakage in the system, there were undoubtedly
more CO_2 radicles per volume in the carbonate solution than Na
radicles, or, in other words, the solution was slightly stronger
than N/100 in CO_2 radicles. This may be explained by supposing
that a certain amount of bicarbonate was present, in spite of every
precaution we had taken to avoid this, or that a slight amount of
CO_2 had been absorbed from the atmosphere although we had endeav-
ored in every way to guard against this also.

The above results show conclusively the accuracy of the apparatus in transferring and absorbing _relative_ amounts of CO_2 . True, some of the proportions (the fourth for instance) are a little exaggerated, but we allow of an error of ±.05 c.c. of an N/100 solution (=.0055 c.c. CO_2, $0°760$ m m.). In addition to this, these results furnish a method of determining the _absolute_ amount of CO_2 contained in a given amount of the solution.

By dividing the figures of the "Na_2CO_3" column into those of the "$Ba(OH)_2$" column and averaging the results, we obtain the factor 1.038. This is the factor by which each c.c. of the Na_2CO_3 solution must be multiplied to get its true CO_2 value in terms of N/100. Its alkali (Na) of course cannot have changed, and is still N/100.--- Taking this factor for the absolute CO_2 strength of each c.c. of the $Na_2 CO_3$ solution, we next conducted the following experiment: The Na_2CO_3 burette was transferred to the first of three absorption chambers, and the system freed of atmospheric CO_2. The mercury level was raised in the first chamber so as to use only the chamber part. An excess of $BaCl_2$ (10 - 14 c.c.) was now made in the chamber by titrating the standard solutions, then a slight excess of $Ba(OH'_2(5 - 7 c.c.)$ added. Next 2 - 4 c.c. of the Na_2CO_3 solution---carefully measured--was dropped in. By this means all of the CO_2 in the latter solution was changed to $BaCO_3$ and precipitated, leaving NaOH and the excess $Ba(OH)_2$ in the solution. These were now neutralized by the HCl and a good reaction obtained with phenolphthalein, because of the excess $BaCl_2$ present.[28] Next a _measured_ excess of the HCl was added to break up the $BaCO_3$. Part of the CO_2 liberated passed over to the second absorption chamber at once, but a considerable portion remained

dissolved in the solution. About five minutes were allowed for the air stream to remove as much of the dissolved CO_2 as possible. Then a <u>measured</u> amount of baryta was added to neutralization. Part of the baryta neutralized the excess HCl, and part the CO_2 in solution. (There was no way of telling where the one left off and the other began, and it is very probable the action was simultaneous). However, as the CO_2 in solution had required its equivalency in HCl to free it, it could be taken as so much HCl remaining in solution since it reads acid to the indicator, the same as any mineral acid. Therefore, the difference between the <u>measured</u> acid and the <u>measured</u> baryta added, or the actual loss in the strength of the acid, was equivalent to the CO_2 which had gone out of the solution and passed over. Or this difference should have been the same as the loss found in the baryta in the absorbing chamber beyond. A sufficient interval (about 10 - 15 minutes) was allowed to complete the transfer of the gas from the large absorption chamber where it was made. Care was taken to ascertain that there was no $BaCO_3$ precipitate in the chamber to start with, and that all of the precipitate formed in the process was again dissolved up by the acid.

The following is a protocol of two experiments:

<center>Exp. 1.</center>

Na_2CO_3 added----------------4.51 c.c.
HCl added (to excess)----10.72 "
Ba(OH')2 added (to neutralize)6.32"

<center>Exp. 2.</center>

Na_2CO_3 added-------------4.44c.c.
HCl added (to excess)--10.53 "
Ba(OH')2 added (to neutralize)8.55"

From this:

The $Na_2 CO_3$ equivalency in N/100-------- ------4.51

The CO_2 equivalency in N/100 was 4.51 X 1.038$=$ 4.67

The HCl to neutralize the Na-------------------$=$ 4.51

Therefore the true excess HCL was 10.72 - 4.51 $=$ 6.21

But the acidity actually found was----------------$=$ 6.32

Therefore the acidity due to the CO_2 remaining in solution was 6.32 - 6.21------------$=$.11

Therefore the CO_2 actually freed and sent over was 4.67 - .11-------------------$=$4.56

The amount of CO_2 caught was$=$4.58

The $Na_2 CO_3$ equivalency in N/100----------------------4.44

The CO_2 equivalency in N/100 was 4.51 X 1.038----$=$ 4.61

The HCl to neutralize the Na----------------------- $=$ 4.44

Therefore the true excess HCL was 10.53 - 4.44 ----- $=$ 6.09

But the acidity actually found was------------------ $=$ 8.55

Therefore the acidity due to the CO_2 remaining in solution was 8.55 - 6.09------------ $=$ 2.46

Therefore the CO_2 actually freed and sent over was 4.61 - 2.46-------------------- $=$ 2.15

The amount of CO_2 caught was$=$ 2.25

Five consecutive experiments with the same solution of Na_2CO_3 gave the following:

Experiment	1.	2.	3.	4.	5.
CO_2 sent over (c.c.N/100 Sol.)	2.15	3.66	4.56	3.55	3.56
CO_2 caught	2.25	3.73	4.58	3.50	3.65

It will be seen that the greatest discrepancy here is .10 c.c. N/100 solution ($=$ .01 c.c. CO_2 , 0° 760 m.m.). When it is taken into consideration that several end reactions and twice as

many burette readings have been involved, it must be admitted that the results are practically exact.

This is probably the most rapid and accurate method known to date for determining small bulks of CO_2. When it exists as a gas, it is simply a question of connecting up its retainer with the absorption spiral. When it exists in solution or in the form of a salt solution, it is freed by strong acid in the special form of Liberator described. When it exists in combination as a solid, it is freed by the process just described for the $BaCO_3$ precipitate, or or by taking a weighed amount in a small tube and dropping into the Liberator.

Some experiments were made to determine how long it re-

Experiments of Special quired to remove all the CO_2 dissolved

Interest in a solution. Several conditions, of course, had to be taken into account, such as reaction and nature of the solution, temperature, speed of air current, etc. The few experiments made along this line seemed to indicate, that, in about twenty minutes at a speed of eight liters per hour and a temperature of about 22° C., all appreciable CO_2 could be removed from 20 c.c. of an N/100 acid solution into which 5 c.c. of an N/100 $Na_2 CO_3$ solution had been dropped. The last traces were of course the most difficult to remove.

Experiments were made to determine the completeness of absorption of large amounts of CO_2 suddenly thrown into the air-stream. As long as the current was no faster than eight liters per hour, absorption was complete even when the loss in the N/55.5 baryta amounted to 5 c.c. in 10 minutes. In other words, it seems that at an eight liter rate every molecule of gas contained in an air bubble must come in contact with the baryta solution in the spiral

before it passes through.

A series of interesting "check" experiments were[was] made on one occasion: The CO_2 from a measured amount of Na_2CO_3 solution was freed in the first chamber. The amount of acid necessary to free it was also recorded[noted]. The gas was caught in the second chamber and estimated. The amount of acid again necessary to free it was recorded and the gas was caught and estimated in the third chamber, and so on through the fourth chamber. Thus each chamber had two records of the same sample of gas. In all of the eight records so obtained the greatest difference was less than 4% of the bulk of the gas handled. When it is taken into consideration that this involved the breaking up of the $BaCO_3$ precipitate on the walls of the spirals (which is no part of the original method for which the apparatus was constructed.) This is remarkably close.

Synopsis of Precautions for Conducting Experiments

Always fill the burettes to the zero mark, and use as near 24 c.c. of baryta as possible. This will lessen the risk of reading the burettes incorrectly.

Never leave a part of a drop hanging on the dropper - always cut off the last drop sharply.

Never overshoot the alkali mark[limit] in titrating; some of the $BaCO_3$ precipitate will be attacked at once and a corresponding amount of acid improperly used up.

Make frequent "no leakage" tests by the methods explained on page 46.

See that all stop-cocks in the air system are well vase-lined and show no "air lines."

After exposing any part of the chamber or spiral to acid or alkali, remember that they must be carefully flushed by a neutral solution before being used again, even though mercury has passed over it and the acid or alkali has been removed.

When the rubber jacket around the bottom of the titration chamber has aged somewhat, it will probably fit loosely and may permit bubbles of air to enter and pass up along the inside of the chamber into the baryta solution. These are easily distinguishable from the bubbles of the air current, coming up from the spiral, by their origin around the edge of the chamber. The loose jacket may then be wired by one round of copper wire placed as near the bottom edge of the chamber as possible(to prevent pockets which might otherwise form and hold back solutions).

When titrating, after an absorption experiment, be sure to flush out the spiral until neutral.

Never let the $BaCO_3$ precipitate collect to any extent in the spiral, because of its power to retain solutions. Break it up with a few c¦c. of the standard acid.

Make titrations every four or five days to note any change in the strength of the baryta solution. These changes will occur generally when the baryta solution is nearing the bottom of the reservoir bottle.

Always discard the solutions which have stood in the burettes or distributing system for two or three days, or more.

Keep the air current going steadily, and at about eight liters per hour.

Never permit more CO_2 to be absorbed in any one period than is equal to about one-fourth of the strength of the baryta solution.

If, by accident, the mercury in the spiral is lowered enough to permit of a few drops back flow of solution from the spiral toward the mercury reservoir, the solution can be redeemed without appreciable loss by at once forcing it back into the spiral with the mercury.

Be certain that nothing but a neutral solution, if any, remains in the inlet tube of the spiral, when ready to take a reading.

When adding solutions, see that they do not splash beyond reach on the walls of the chamber.

Keep the walls of the burettes clean.

Watch for leaky stop-cocks on the burettes. Leaks are apt to be shown in any one of three ways: (1) an actual dripping from the stop-cock: (2) a very slow drop of the meniscus in the burette- this may only be discernable by reading the level at intervals of a half hour or more: (3) a gradual increase in the size of the drop hanging on the end of the dropper. If this latter occurs without any change in the meniscus level in the burette, there is a leak through the stop-cock from the distributing system only.--As a full drop equals .07 c.c., this hanging drop may be estimated for the time being.

The greatest error in reading the burettes should not exceed .025 c.c.

The end reaction should be estimated to within half a drop = .035 c.c.

By the laws of probability and chance, the total reading errors -- burettes, hanging drops, and end reactions -- should about balance up in each titration and leave no error worthy of account from these sources at all.

Experiments on the CO_2 Yield from Isolated Muscles

A full protocol of each experiment is given later. We will here only group the experiments and discuss them according to their objects and results.

The first series of experiments was to determine the amount and rate of CO_2 evolution from isolated muscle at rest. These experiments were conducted on both frog and mammalian muscle.-- We have adopted Fletcher's method[29] for the graphic representation of each experiment. The plate accompanying each protocol of an experiment practically explains itself. It will be noticed that the tops of the rectangles, constituting the CO_2 output, form an irregular curved line and in some of the plates this curved line has been drawn; i.e., where it is thought to be more explanatory. The absence of a rectangle in the course of a curve means that the CO_2 for that period was not recorded.-- It may be said of all the experiments that they show a relatively large yield of CO_2 immediately after excision, the rate of which gradually decreases during the hours which follow. Certain irregularities will be discussed in their proper place.

When the rate of yield from normal <u>resting</u> muscle is represented graphically by a line, the latter takes the form of a concave curve, most pronounced during the first hours after excision. (Exp. 1 - 9, 13 $\div$ 15, 21, 25).

The rate of removal of the CO_2 from the muscle chamber, or variations in the speed of the air current seem to have no influence whatever on the rate of the CO_2 yield, providing, of course, the current does not stop altogether. (Exp. 1 - 9).

With frog's muscle the decline in rate of yield proceeds
Frog's Muscle   rapidly until about the third hour, when, in nearly
  at Rest   all of the experiments, the curve takes a more hori-
                   zontal direction for an hour or two, producing a
"shelf" in the curve in most instances (Exp. 1, 5 - 7, 21, left leg,
25), and an actual rise in other instances.   (Exp. 2, 4, 12 --
right leg-- 21, right leg).   This rise, however, never approaches
the yield of the first hour.   Between the fourth and sixth hours,
varying with different muscles, there is a second more or less
pronounced drop in the curve of discharge in nearly every case. At
about the sixth hour, the curves show the greatest inconsistency in
the directions which they are apt to take.   In some cases there is
a continuation, even more marked, in the rate of fall of the curve
(Exp. 4, 21 -- two examples), while in others there is an abrupt
rise, marking the largest "hump" shown in the curve.   (Exp. 5 - 7).
After the seventh hour, unless putrefactive changes appear at once,
the curves again assume one general direction, generally continuing
horizontally for two or three hours, thus producing a "plateau",and
then beginning a gradual decline.   Or this decline may begin at once
after the seventh hour.   (Exp. 5, 21 -- two examples).   The curve
continues this direction until putrefactive processes in the muscle
set in, which is evinced by a rapid rise in the curve.   (Exp. 6 -
7 and perhaps 21).--- With the exception of experiments 21 and 25,
the temperature during the rest of this class of experiments ranged
between 19°- 25° C.   For those two experiments, it was 29°- 30° C.
The variations in temperature, within the limits noted above, seem
to have had no effect upon the curve.

Mammalian Muscle
at Rest

With mammalian muscle, the irregularities in the CO_2 evolution noted in frog's muscle, at least in five experiments (Exp. 8, 9, two samples each, and 15) do not appear, but the curve has a general and regular concave direction; the decline in the rate of yield, however, becoming less and less up to ten hours after excision. Only two muscles were followed beyond this, but putrefactive changes then set in, giving the characteristic sudden rise in the curve. (Exp. 9).

The influence of contraction upon the CO_2 yield from normal Contraction excised muscle was followed in a total of seventeen experiments, and with startling variations in the results. (Exp. 10 - 12 -- two examples each,-- 13 - 17, 19, 20, 22 - 25). All these experiments were carried out within a few hours after excision, and great care was taken not to fatigue the muscles, either by strong or continued stimulation, (which, Fletcher claims, produces an extra yield of CO_2[29]), and thus drive them into premature rigor mortis, -- a condition in which it is generally acknowledged more CO_2 is evolved.[7] The plan followed in order to avoid fatigue was to stimulate the muscles, either directly or through the nerve, by an induced current of just sufficient strength to cause a good contraction, -- the current made and broken by the interposition of a metronome with mercury contacts. The general rule was to give the shocks at intervals of about four seconds for a period of five minutes, making thus seventy-five contractions in that time. The coil of the secondary current was started at a good height

from that of the primary current, according to the irritability
of the muscle, and gradually lowered a few centimeters whenever
the contractions became feeble.   Next, a five minute rest inter-
val was allowed the muscle, after which it usually evinced signs
of renewed irritability, so that the secondary coil could be
raised slightly to start the next series of contractions.   In
this way, three contraction periods of five minutes each would be
given in a half hour's time, or six in an hour's time, according
to the bulk of the muscle and its sensitiveness to stimulation.
Generally a long rest period of half an hour to an hour preceded
and followed each half hour or hour of contraction.   By this
means,a muscle could be made to contract within the first hour or
two after excision -- long before rigor mortis ordinarily sets
in -- and at the same time rest intervals were frequent enough to
forestall fatigue.   As a record was kept of the yield during the
half hour or hour period before contraction and similarly after
contraction, a direct comparison with the "normal curve" for that
time is possible;   for, during the first three or four hours the
curves of resting muscles deviate from each other very little (see
page 60   ).   But better still, the contracting period of one
muscle is generally compared directly with the chronologically
identical period of the homologous muscle of the same animal at
rest.

      With frog's muscle, the influence of contraction upon the

The CO_2 Output from Con- CO_2 evolution gave two classes of

  tracting Frog's Muscle   results, -- the one in which a

marked increase was found for every period of contraction (Exp.
10 - 14, 17, 20, 24 third period), and the other in which contrac-

tion seems to have had very little influence whatever upon the CO_2 . (Exp. 12 -- right leg, 22, 23, 24- seventh period, 25).

The contradiction in these two classes of results requires careful scrutiny of the conditions under which the muscles existed, and we are by no means yet able to affirm positively that we have reached the right conclusions. As before cited, experiments 1 - 9 show that the limited variations in the rate of the air current, used to remove the CO_2 evolved, have no influence upon the yield (unless the current is stopped, when there is at once a rapid decline in the yield). The rate of current used throughout all of the experiments was kept approximately the same, consequently this factor, as an influencing condition, can be eliminated. The temperature changes recorded in the "normal curve" experiments 1 - 9 and 21, ranging from $19^{\circ} - 31^{\circ}$ C. also seem to have had no effect. As all our experiments were conducted between the temperatures $18^{\circ} - 33^{\circ}$ C, and the greatest temperature variation in any one experiment was less than five degrees, temperature within these limits as an influencing factor can be disregarded. Although no absolutely accurate record was kept of the amount of negative pressure to which the muscles were subjected, it was always between 20- 30 m.m. of mercury. As the negative pressure varied nearly proportionately with the speed or pull of the current, and as a record of the latter shows no detrimental influences, this may be also eliminated. The muscles were always excised as soon as possible after death; i.e., before the stoppage of circulation, and in practically the same manner. Each was moistened by NaCl solution (.60 or .75%) before enclosing in the chamber, and retained its moist condition perfectly. Hence

the factor of the preparation and preservation
of the muscle may be excluded. In some cases contraction was
produced by direct stimulation to the muscle and in others by
stimulation through the nerve. In the experiments by nerve stim-
ulation, some of them showed either a slight increase in the rate
of the CO_2 yield (Exp. 20, right gastrocnemius) or a check in the
ordinary decline to be expected (Exp. 17); while others showed no
increase at all (Exp. 22 - 25). Those experiments in which con-
traction was by direct stimulation may be similarly divided: In
experiments 10, 11, 12 -- left leg, 13, 14, 18 last period
(curarized) there was a marked increase in the yield; in experi-
ments 12 right leg, and 25 there was no increase. No difference,
therefore, was observed in the results on uncurarized muscle,
whether stimulated directly or through the nerve.

The muscles in all cases were unweighted, but an examina-
tion of the protocols reveals the following facts which show that
the work done under different conditions might possibly enter as a
factor: In some of the experiments the contracting muscle had
nothing to move but its own mass. Such cases are, for instance,
those in which isolated gastrocnemii were used. (Exp. 14, 20,
22 - 25). In others, the whole leg and thigh, without the feet,
were used, and upon contraction, the limb would straighten at the
knee thus raising the weight of the thigh. (Exp. 10 - 13, 17,
18). In other experiments, again, the muscles took such a posi-
tion in the chamber as to lie between the inlet tube and the cham-
ber wall so that in contracting there was a slight amount of fric-
tion between the muscle and the glass -- (Exp. 10, 11, 12 -- right
legs -- 13 (?), 17 (?). In all of the cases in which any of these

conditions were noted, the yield of CO_2 was higher than in corresponding experiments in which this slight additional work was absent.   Hence the question of work performed by a contracting muscle appears to be an important factor in its CO_2 evolution. If a freshly isolated muscle is supplied with enough pure air, there can be no doubt that an increased yield of CO_2 may be obtained during a period of contraction, and without driving the muscle into fatigue, but our experiments seem to indicate that at least the one condition necessary is that the muscle shall perform some work.   The relation between the CO_2 yield and the amount of work performed has not been studied by us, but it offers a new and interesting field for investigation.

Three experiments on contraction were made with mammalian

Mammalian Muscle muscle.   (Exp. 15, 16, and 19).   In experiment

Contracting   15, the gastrocnemius of a nine week's old pup was stimulated directly, with a very striking increase in the amount of CO_2 obtained during the period of contraction.   The protocol shows also that friction caused the muscle to perform some work.   In experiment 16, with a dog's gastrocnenii, exactly similar results were obtained for both muscles, under the same conditions of work;   and experiment 19, with the gastrocnemius of a cat, yielded the same results.   There were no experiments with mammalian muscle in which they performed absolutely no work during contraction, hence a comparison of that sort cannot be made, but a comparison may be made with experiments 8 and 9 in which four curves of mammalian muscle at rest are shown.   Such a comparison can leave no doubt in the mind of the reader that contraction in mammalian muscle, although perhaps modified by work

limitations, has the effect of increasing the CO_2 output.   (See previous paragraph for the effect of friction).   The greatest care was also taken in these experiments to preclude fatigue.

The experiments involving the use of curare to paralyze
Curare   the motor nerve endings in muscle in order to study the influence of subsequent nerve stimulation on the CO_2 yield from such muscle may also be grouped in two classes: frog's and mammalian muscle.

The main difficulty in conducting experiments upon curarized muscle is to ascertain whether the nerves are irritable to the electric shock given them.   The muscles, of course do not contract in response to the stimulation of the nerve.   However, as stimulation of muscle through the nerve requires a much weaker current than when stimulated directly, one method is to conduct the experiment upon curarized muscle and nerve in an exactly similar manner to a contraction experiment upon normal muscle; i.e., by starting with a weak current and gradually increasing its strength as the nerve becomes less irritable.   Another method is to curarize one muscle and to stimulate its nerve exactly the same as the nerve of the homologous muscle non-curarized.   In these experiments we have assumed that the irritability of each nerve is about the same, so that the same strength of current can be used upon the nerve to the curarized muscle as is found necessary to produce contraction in the normal muscle, during the corresponding period after excision.

The work on frog's muscle is shown in experiments 14,17,18,
Curarized Frog's Muscle.   20 and 24.   The method of curariz-
  Nerve Stimulation   ing and comparing the homologous

muscles is explained in each protocol. Eliminating experiment
14 (see protocol), it will be seen that a check in the regular
decline of the curve was noted in experiment 17 and an actual rise
in experiment 20. On the other hand, experiments 18 and 24, (ex-
cluding last three periods of 24), both conducted under as nearly
similar conditions as possible to 17 and 20, exhibit no change
whatever in the normal fall of the curve. -- Although four ex-
periments is too small a number from which to draw positive con-
clusions, especially in the face of the difficulty above mentioned,
it may be said that the influence of curare is an uncertain one
upon the CO_2 output from frog's muscle during periods of nerve
stimulation, and probably depends upon several conditions not yet
noted in our experiments.

The work on mammalian muscle is shown in experiment 19.
<u>Curarized Mammalian Muscle.</u> Here there can be no disputing the
<u>Nerve Stimulation</u> fact that nerve stimulation to the
curarized cat's gastrocnemius resulted in a large increased yield
of CO_2 .

This experiment was carried on for several hours after the
contraction periods. The curve representing the curarized mus-
cle, it will be noted, after the first hour and a half, maintained
a nearly constant level up to seven hours. That from the non-
curarized muscle began a gradual ascent, one-half hour after the
stimulation period, so that the yield of the last period was more
than that of any of the initial periods. Whether this was the
result of putrefactive processes taking place very slowly, or real-
ly represents the increased CO_2 output due to the onset of <u>rigor</u>
<u>mortis</u> (a possible explanation), cannot be said. The period of

contraction immediately preceding would undoubtedly result in hastening rigor in the muscle.

Experiment I *Feb. 25,*

To Determine: Amount and rate of CO_2 evolution from Frog's gastrocnemius at rest.

Method: Ordinary sized frog killed by pithing at 4:00 P.M. Right gastrocnemius hung in muscle chamber "B" (capacity 65cc) at 4:25. Air current started at once and continued until 4:28 to remove atmospheric CO_2. Then switched to absorption chamber III.

Data: A few moments after starting, the water pressure upon which the air current depended, suddenly fell and remained low until 7:30. During this interval the gases evolved were removed by occasionally forcing air through the whole system. However the figures for this interval should not be taken as typical.

Time	CO_2 Obtained	CO_2 rate per $\frac{1}{2}$ hour	Air Current rate per hr	Temperature
P.M.				
4:28-5:00	.05 cc	.05 cc	No current	25° C
5:00-5:30	.04	.04	" "	25
5:30-7:30	(.10)	(.025)	" "	24
7:30-8:00	----	----	----	23
8:00-9:00	.06	.03	10 liters	22
9:00-10:00	.06	.03	10	22-21
10:00-11:00	.06	.03	6.25	21-20.5

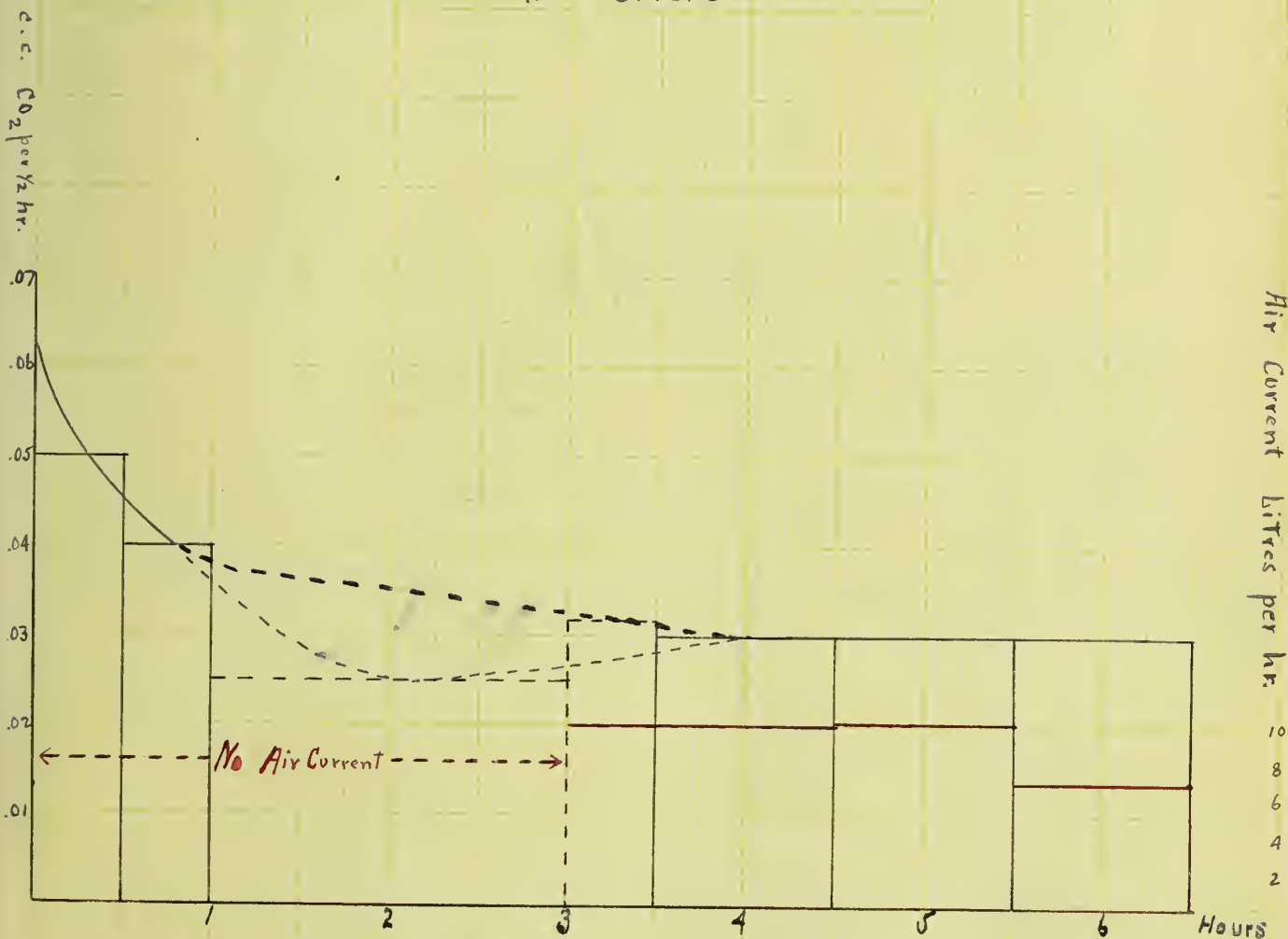Discontinued. Muscle very moist and to all appearances in perfect condition.

Plate 1.

Curve of CO_2 obtained per ½ hr. from frog's <u>gastrocnemius</u> at rest

Rectangles — volumes of CO_2 per period included

Heavy dotted line — probable direction of curve had air current
been maintained.

Fine dotted line — direction which curve took

Red lines — rate of air current

Experiment 2 $\widehat{f}$ ʌᴎ ᴢ6

To Determine: Amount and rate of CO_2 evolution in frog's gastrocnemius at rest.

Method: Left gastrocnemius of same frog killed for Experiment 1. The frog had been kept in water. Sciatic nerve and circulation still intact. Muscle excised 11:10 A.M. Placed in muscle chamber "B" at 11:15. Current started at once; switched to absorption chamber III at 11:20.

Data:

Time	Hours from start	CO_2 obtained	CO_2 rate per $\frac{1}{2}$ hr	Air current rate per hr	Temperature
A.M.					
11:20-12:20	1	.05 cc	.025 cc	7.7 liters	18° C
P.M.					
12:20- 1:20	2	.03	.015	10.5	19
1:20- 2:20	3	.03	.015	9.0	19
2:20- 3:20	4	.04	.020	8.5	19
3:20- 4:20	5	.04	.020	9.5	19
4:20- 5:20	6	?	?	9.8	19

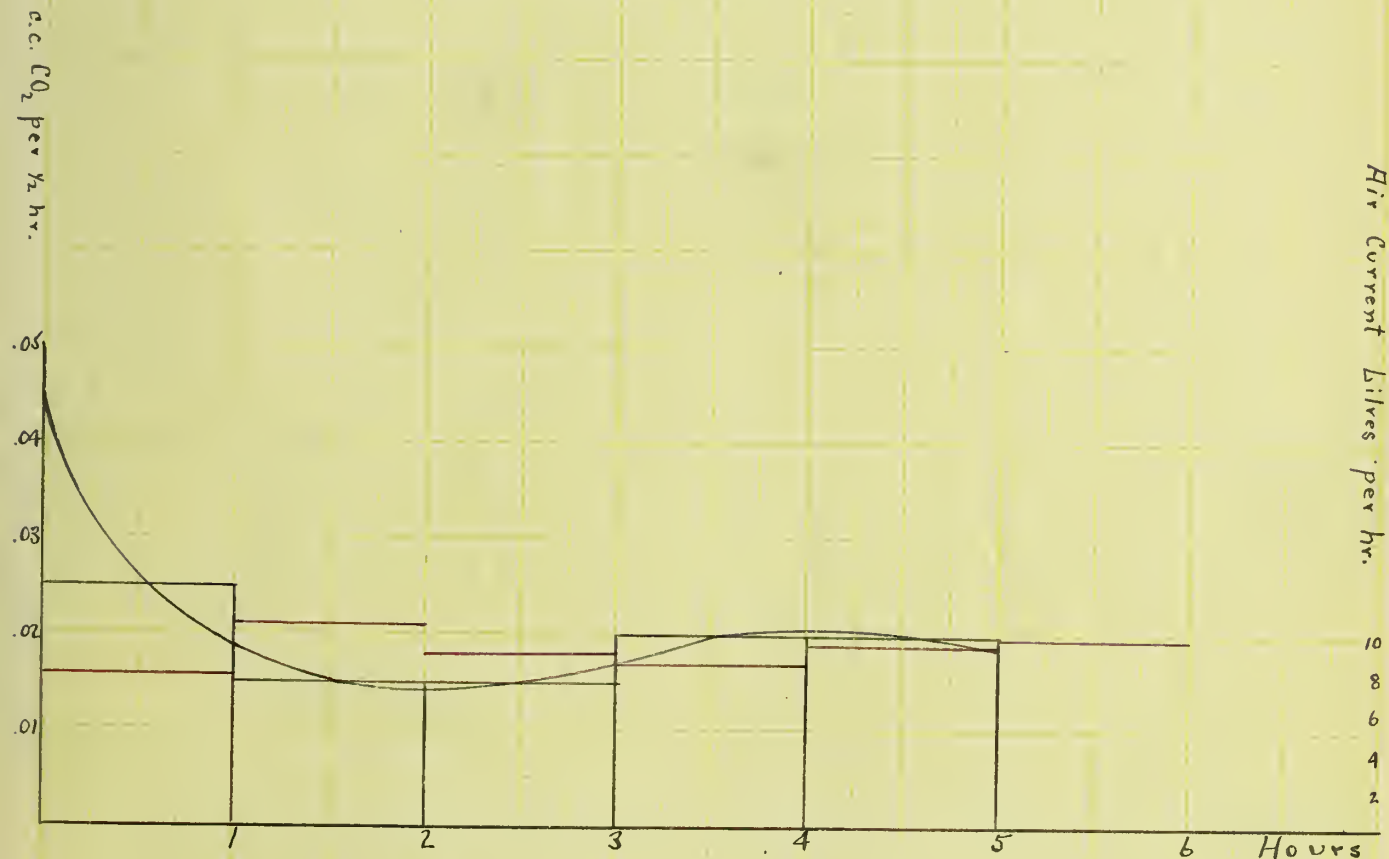The experiment was ended by an accident at 5:20. Muscle appeared in perfect condition upon removal.

Plate 2

Curve of CO_2 obtained per ½ hr. from frog's gastroenemius at rest

Rectangles — volumes of CO_2 per period included

Red Lines — rate of air current

Experiment 3   *Feb. 25*

To Determine: Amount and rate of CO_2 evolution from frog's en-
tire leg at rest.

·Method: Ordinary frog killed at 6:58 P.M. Left leg severed
at pelvis, keeping thigh muscles as intact as possible, skinned,
and foot removed at ankle. Placed in muscle chamber "B" at 7:23
and air current started; switched to absorption chamber III at 7:30.

Data:

| Time	Hours from start	CO_2 obtained	CO_2 rate per $\frac{1}{2}$ hr	Air current rate per hr	Temper- ature
P.M.					
7:30- 8:30	1	.24 cc	.12 cc	7.25 liters	20.5° C
8:30- 9:30	2	.17	.085	9.5	20.5
9:30-10:30	3	.13	.065	7.25	20

Discontinued. Muscles appeared in perfect condition.

Plate 3

Curve of CO_2 obtained per ½hr from frog's leg at rest

Rectangles — Volumes of CO_2 per period included

Red Lines — Rate of Air Current

c.c. CO_2 per ½ hr.

Air Current Litres per hour

Hours
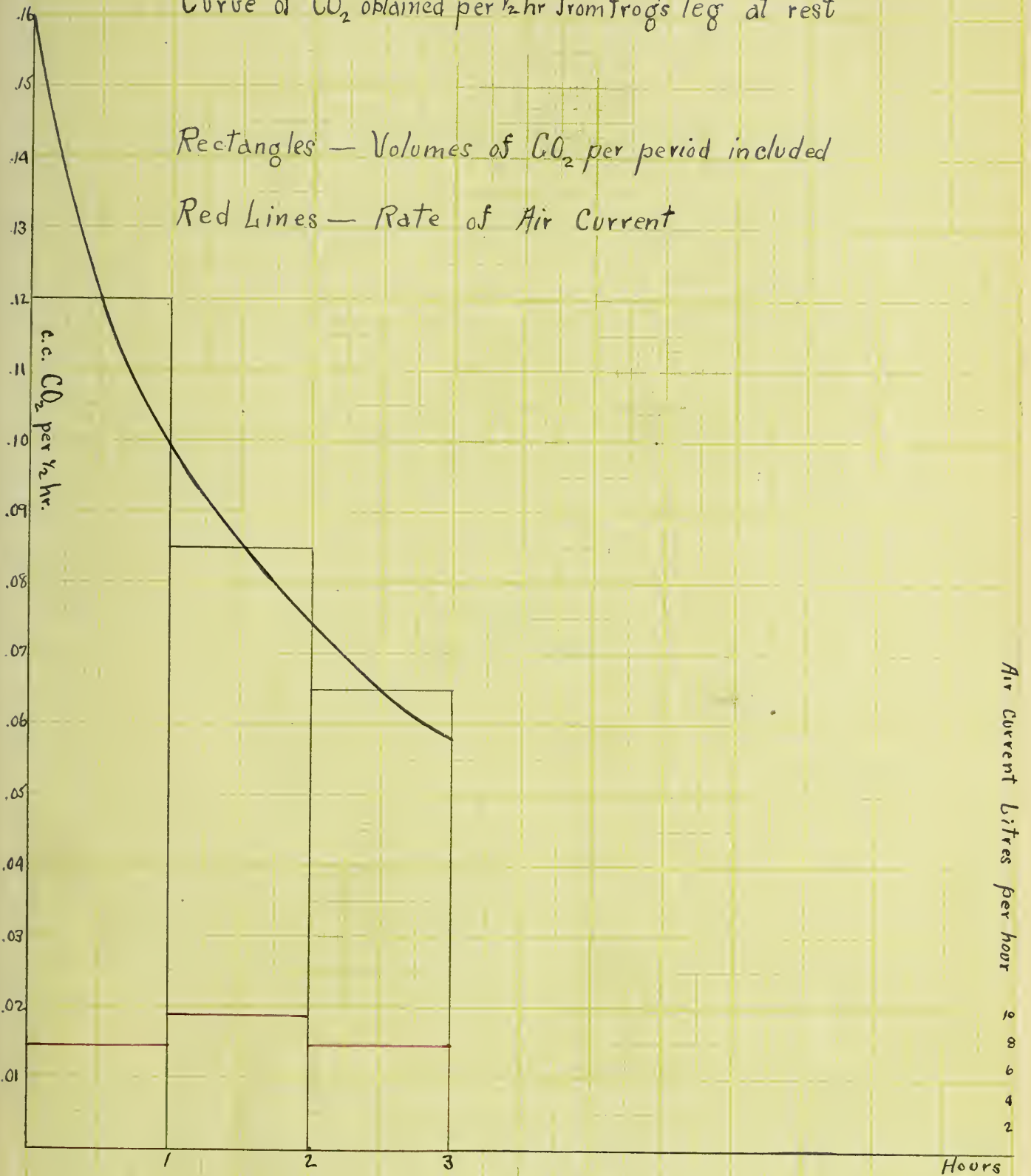
Experiment 4

To Determine: Amount and rate of CO_2 evolution from frog's entire leg at rest.

Method: Right leg of frog separated at pelvis, keeping thigh muscles as intact as possible, skinned, and foot removed at ankle, 8:27 A.M. Leg placed in muscle chamber at 8:45.

Data:

Time	Hours from start	CO_2 obtained	CO_2 rate per hour	Air current rate per hr	Temperature
A.M.					
8:45- 9:25	2/3	.06 cc	.09 cc	7.00 liters	19°C
9:25- 9:45	1	.03	.09	9.75	19
9:45-10:45	2	.08	.08	8.66	19
10:45-11:45	3	.09	.09	8.00	19.5
11:45-12:48	4,3min.	.08	.08	8.66	20
P.M.					
12:48- 1:45	5	.07	.07	8.33	20
1:45- 2:45	6	.07	.07	7.00	20
2:45- 4:00	$7\frac{1}{4}$	.07	.06	7.50	20
4:00- 4:45	8	.04	.06	7.50	19.5
4:45- 5:45	9	.06	.06	5.66	19
5:45- 8:45	12	.15	(Current stopped $\frac{1}{2}$ hr)		19
8:45- 9:45	13	.06	.06	6.66	19

Leg removed. Muscles had gone into rigor. In very moist condition. Accuracy of above data was afterward found to be in doubt because of possible variable error in method. However the general direction of curve on opposite page may be regarded as typical.

Plate 4.

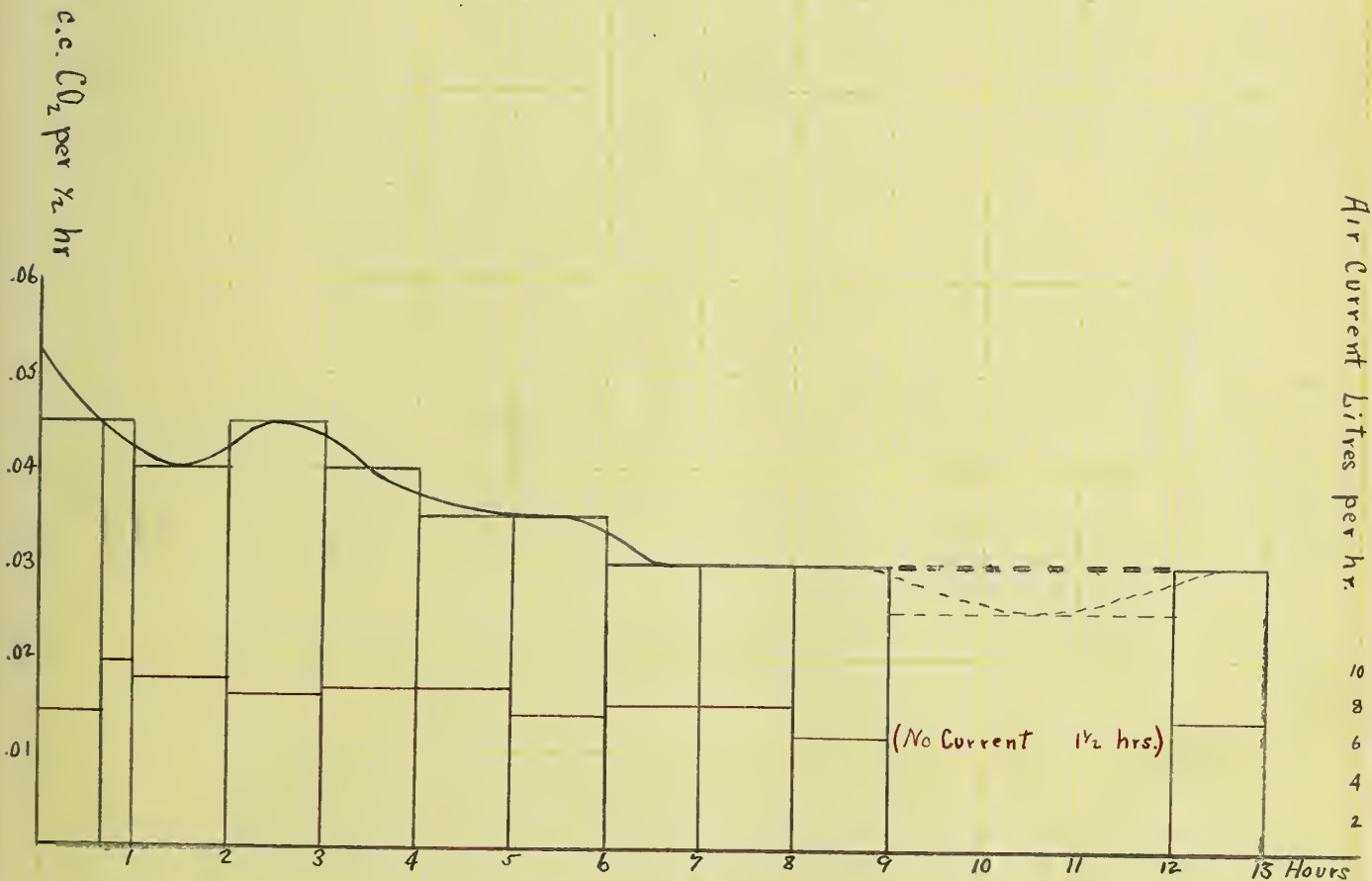Curve of CO_2 obtained per ½ hr from frog's leg at rest

Rectangles — volumes of CO_2 per period included

Heavy Dotted Line — probable direction of curve had Air
  Current been maintained

Fine Dotted Line — direction which curve took

Red Lines — rate of Air Current

(No Current   1½ hrs.)

Experiment 5

To Determine: Amount and rate of CO_2 evolution from frog's entire leg at rest.

Method: Rather large sized frog killed at 8:30 A.M. Left leg skinned, severed at ankle and pelvis, keeping thigh muscles as intact as possible; placed in muscle chamber "A" (capacity 100 cc) at 8:47. Air current started at 8:48. Correction made for atmospheric CO_2 (=.05 cc) in first period estimation.

Data:

Time A.M.	Hours from start	CO_2 obtained	CO_2 rate per $\frac{1}{2}$ hr	Air current rate per hr	Temperature
8:48- 9:18	0.5	.30 cc	.30 cc	7 liters	19° C
9:18- 9:48	1	.23	.23	8	
(Estima-	2	.30	.15	8	
tions	3	.25	.125	7	
hourly	4	.23	.115	6.6	
until	5	.20	.10	8.4	
10:48 P.M.)	6	.206	.103	7.6	
	7	.226	.113	9.2	
	8	.20	.10	7	
	9	.18	.09	7	
	10	.14	(Current stopped 3/4 hr)		
	11	.18	.09	5	
	12	.176	.088	9	
	13	.171	.086	7.4	
	14	.171	.086	8.5	

Discontinued. Muscles rigid; very moist. No putrefactive smell.
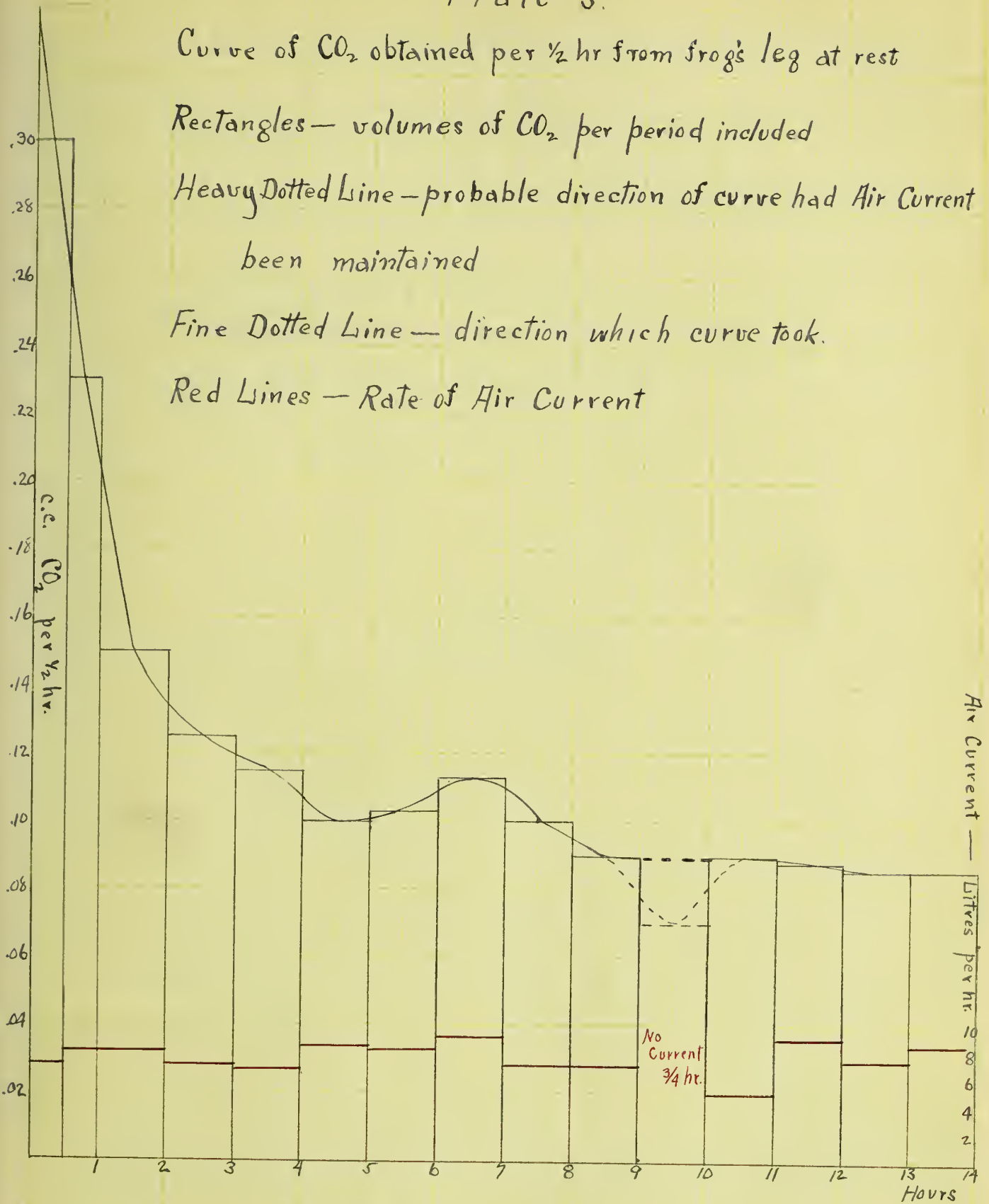
Plate 5.

Curve of CO_2 obtained per ½ hr from frog's leg at rest

Rectangles — volumes of CO_2 per period included

Heavy Dotted Line — probable direction of curve had Air Current

been maintained

Fine Dotted Line — direction which curve took.

Red Lines — Rate of Air Current

Experiment 6

To Determine: Amount and rate of CO_2 from both legs at rest.

Method: Medium sized frog killed, 8:35. Both legs skinned, severed at pelvis and ankles; placed in muscle chamber "A" and current started, 8:49, to absorption chamber II. Correction made for atmospheric CO_2 in first period estimation. Temperature 19° C.

Data:

Time	Hours from start	CO_2 obtained	CO_2 rate per $\frac{1}{2}$ hr	Air current rate per hr
A.M.				
8:49- 9:19	0.5	.286 cc	.286 cc	9.6 liters
9:19- 9:49	1	.164	.164	7.6
(Estima-	2	.276	.138	7.6
tions	3	.226	.113	8.6
hourly	4	.216	.108	8.6
until	5	.190	.095	9.0
10:49 P.M.)	6	.200	.100	8.8
	7	.240	.120	7.6
	8	.200	.100	8.2
	9	.200	.100	8.6
	10	.200	.100	6.6
	11	.172	.086	6.3
	12	.176	.088	7.8
	13	.170	.085	7.4
(Left to run over night	14	.184	.092	8.4
until 8:49 A.M.)	15-24	2.456	(.123)	(40.0 . passed through, but not going next A.M.)
A.M.				
8:49- 9:49	25	.810	.405	8.0
9:49-10:49	26	.860	.430	7.6

Legs had a distinct putrefactive smell on removal. Quite rigid, moist. Putrefaction probably began with increased output of 14th hour.
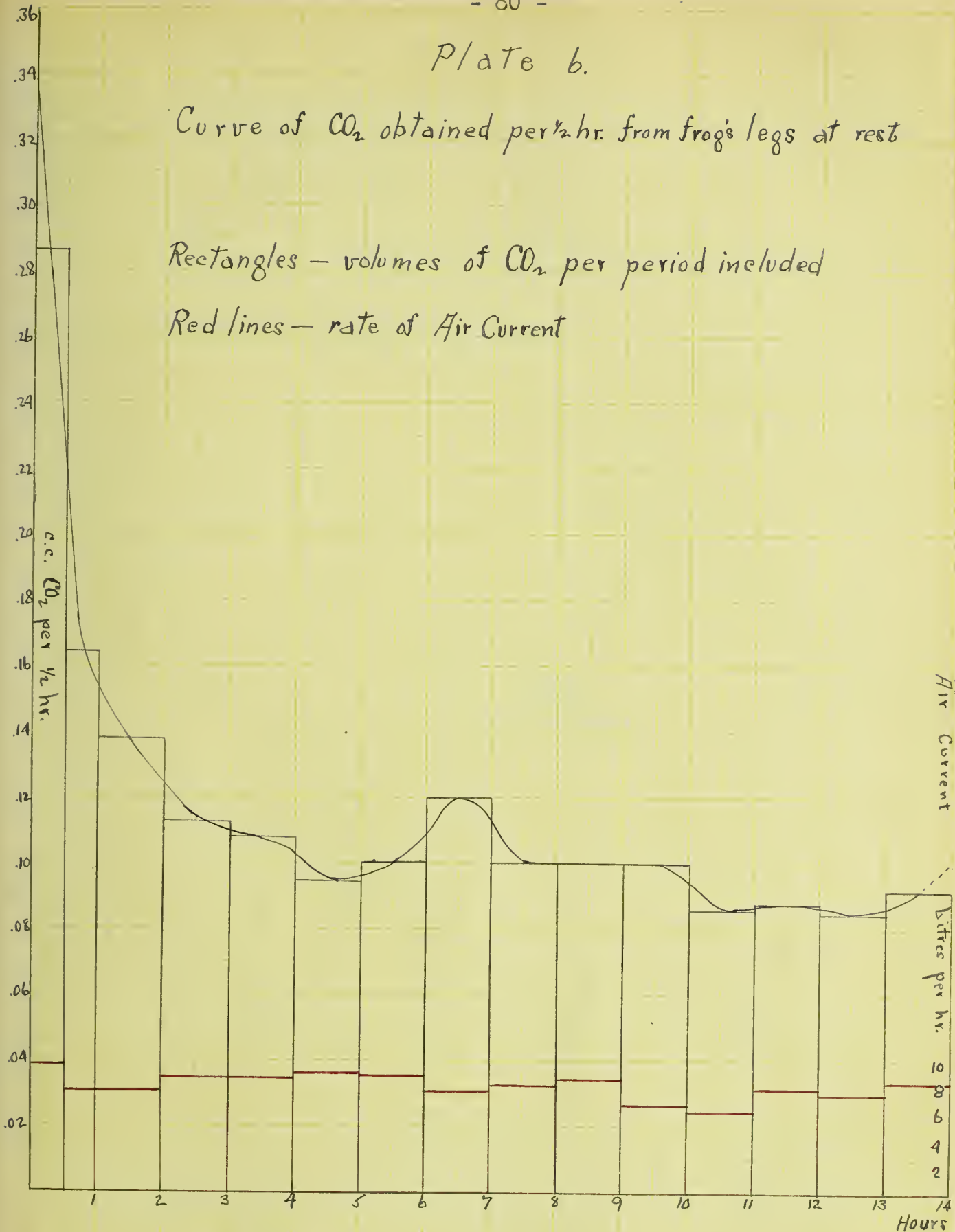
Plate b.

Curve of CO_2 obtained per½hr. from frog's legs at rest

Rectangles — volumes of CO_2 per period included

Red lines — rate of Air Current

Experiment 7

To Determine: Amount and rate of CO_2 evolution from both legs of frog at rest.

Method: Medium sized frog killed at 8:37. Both legs skinned, severed at pelvis, (with as little injury to thigh muscles as possible) and at ankles. Placed in chamber "A" at 8:53 and current started at once to absorption chamber I. Atmospheric CO_2 in chamber corrected for in first period estimation.

Data:

| Time	Hours from start	CO_2 obtained	CO_2 per ½ hr	Air current rate per hr	Temperature
A.M.					
8:53- 9:23	0.5	.286 cc	.286 cc	9.2 liters	23° C
9:23- 9:53	1	.206	.206	10.0	23
(Estima-	2	.238	.169	7.6	23
tions	3	.300	.150	8.6	23
hourly	4	.280	.140	9.3	24
until	5	.282	.141	9.5	24
10:53 P.M.)	6	.246	.123	8.2	23.5
	7	.268	.134	7.1	23.5
	8	.258	.129	7.7	23.5
	9	.286	.143	7.8	24
	10-11	.586	.146	6.4	24
	12	.304	.152	6.8	24.5
	13	.304	.152	8.4	24.5
	14	.354	.177	8.0	24.5
P.M.					
10:53-11-23	14.5	.186	.186	10.6	24.5

Legs removed. No putrefactive smell appreciable. Not rigid. No response to mechanical stimulation of nerves.

Plate 7.

Curve of CO_2 obtained per ½ hr from frog's legs at rest

Rectangles — volumes of CO_2 per period included

Red Lines — rate of Air Current
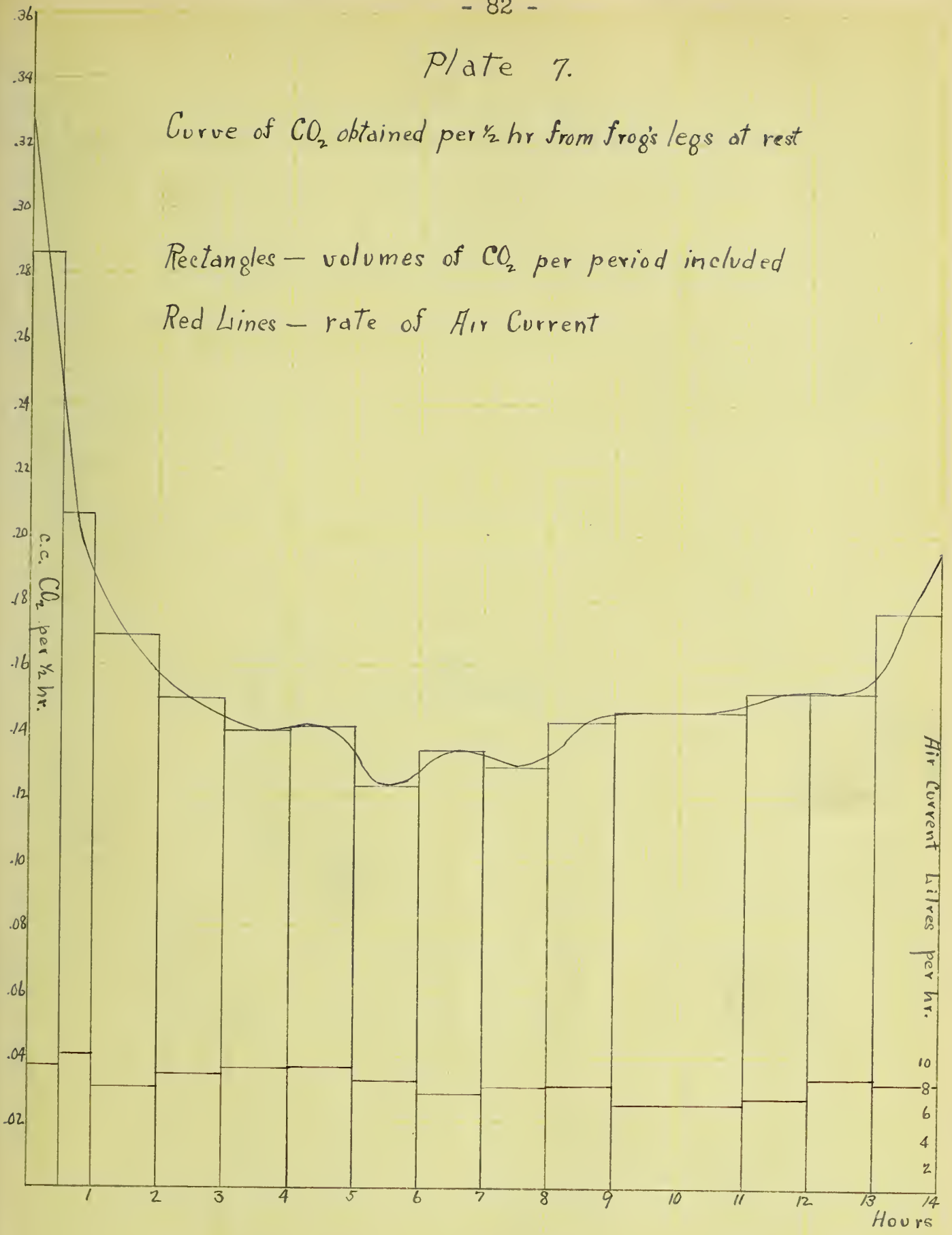
Experiment 8.

To determine amount and rate of CO_2 evolution from legs (of pup) at rest compared simultaneously.

Method: Pup five weeks old, killed at 7:35 P. M. Left hind leg skinned (somewhat lacerated), placed in chamber A at 7:50. Right leg placed in B at 8:02 1/2. Feet had been amputated at ankles. Left leg slightly smaller than right. Neither weighed.

Data:

Time Left	Right	Hours from start	CO_2 obtained L.	R.	CO_2 per 1/2 hr. L.	R.	Air used L.	R.	Temp. same for both
7:50-8:20	8:02½-8:32½	1/2	1.056cc	.922cc	1.056cc	.922cc	4.0L	4.0L	24°C
8:20-8:50	8:32½-9:02½	1	.630	.626	.630	.626	3.8	5.6	24
Hrly until 2:50 A. M.	Hrly until 3:02½ A.M.	2	.990	1.000	.495	.500	6.3	6.4	23½
		3	.740	.792	.370	.396	8.1	5.3	22
		4	.596	.624	.298	.312	8.5	8.	22
		5	.520	.586	.260	.293	8.7	8.	22
		6	.484	.526	.242	.263	7.3	7.3	21½
		7	.442	.490	.221	.245	8.8	9.9	21½
A.M. 2:50-3:30	3:02½-3:42½	7 2/3	[.284]	----	.213	----	5.3	4.5	21½
3:30-4:30	3:42½-4:42½	8 2/3	.400	.446	.200	.223	7.9	6.	--
4:30-5:30	4:42½-5:42½	9 2/3	.366	.400	.183	.200	7.8	4.5	19

On removal, both legs had perfectly sweet smell. No response to direct stimulation by induced current. Left leg not rigid; right leg possibly slightly so. Atmospheric CO_2 corrected for in first period.
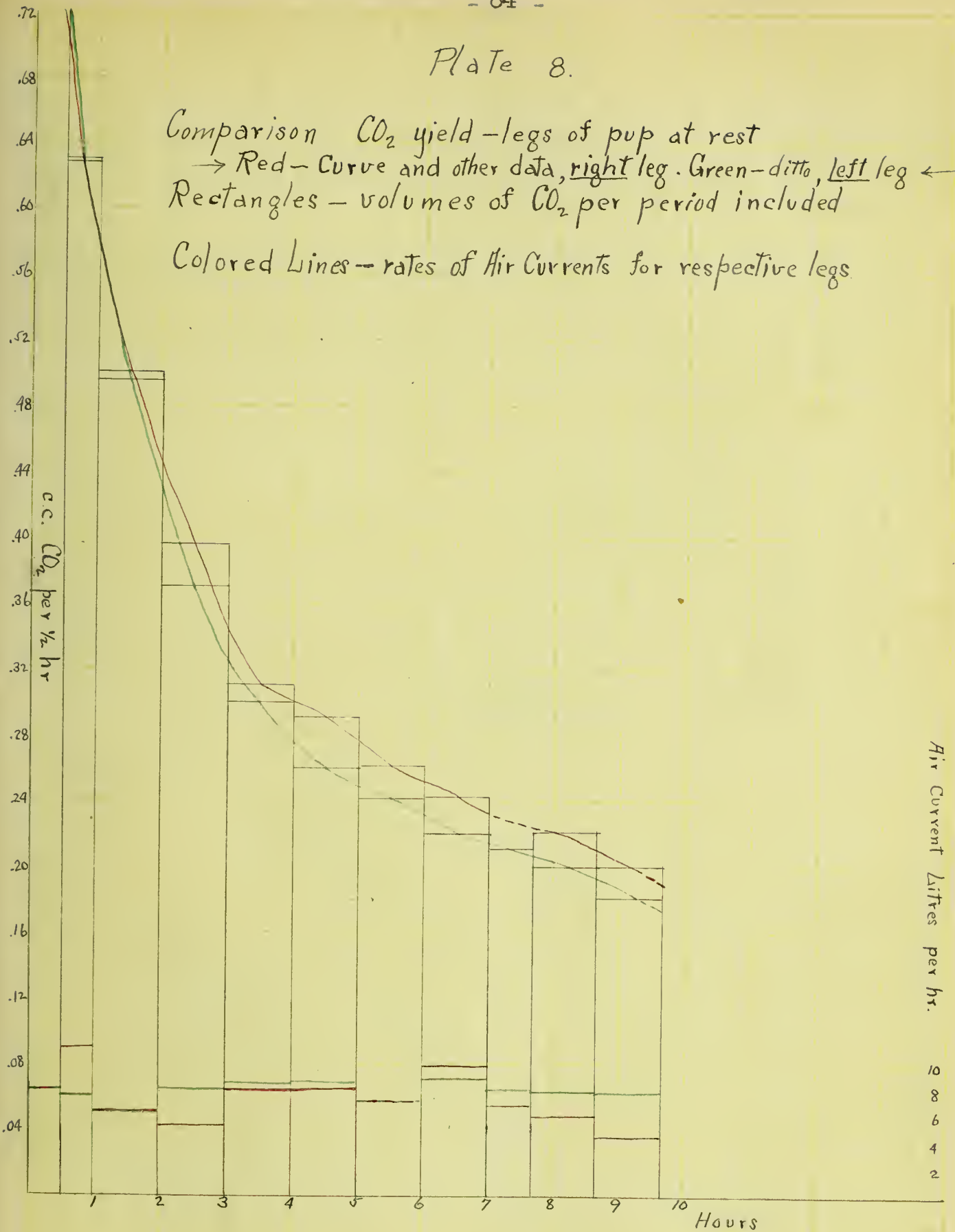
Plate 8.

Comparison CO_2 yield — legs of pup at rest
→ Red — Curve and other data, <u>right</u> leg. Green — ditto, <u>left</u> leg ←
Rectangles — volumes of CO_2 per period included

Colored Lines — rates of Air Currents for respective legs.

c.c. CO_2 per ½ hr

Air Current Litres per hr.

Hours

Experiment 9.

To Determine amount and rate of CO_2 evolution from legs (of pup) at rest, compared simultaneously.

Method: Pup, five weeks' old, killed at 9:20 A. M. Left hind leg skinned(somewhat lacerated) placed in chamber A at 9:32½ Right leg (nearly perfect) placed in B at 9:43.   Feet had been amputated at ankles.   Weight of left leg 29 gms., right 27.4 gms.

Data:

Time Left	Right	Hours from start	CO_2 obtained L.	R.	CO_2 per 1/2 hr. L.	R.	Air used L.	R.	Temp. same for both
9:32 1/2-10:02½	1/2		1.260cc	1.170cc	1.26cc	1.170cc	5.4L	3. L	21°C
10:02 1/2-10:32½	1		.770	.830	.77	.830	5.4	4.5	"
Estimations	2		1.270	1.210	.635	.605	8.5	6.9	"
hourly until	3		1.100	1.080	.550	.540	7.2	10.6	"
P. M. L1:32 1/2 1:43	4		.994	.960	.497	.480	9.4	8.6	20 1/2
L1:32 1/2-3:32 1/2 R1:43 -3:43	5-6		1.724	1.650	.431	.412	14.	14.5	21 1/2
L3:32 1/2-4:32 1/2 R3:43 -4:43	7		.750	.784	.375	.392	11.	7.	"
L4:32 1/2-5 32 1/2 R4:43 -5:43	8		.708	.710	.354	.355	10.2	6.4	"
L5:32 1/2-7:32 1/2 R5:43 -7:43	9-10		1.410	1.400	.352	.350	14.5	17.	21
L7:32 1/2-9:32 1/2 R7:43 -9:43	11-12		1.608	1.670	.402	.417	13.	14.5	"
L9:32 1/2-10 32 1/2 R9:43 -10:43	13		.960	1.080	.480	.540	6.2	7.1	20 1/2
			12.554	12.544					

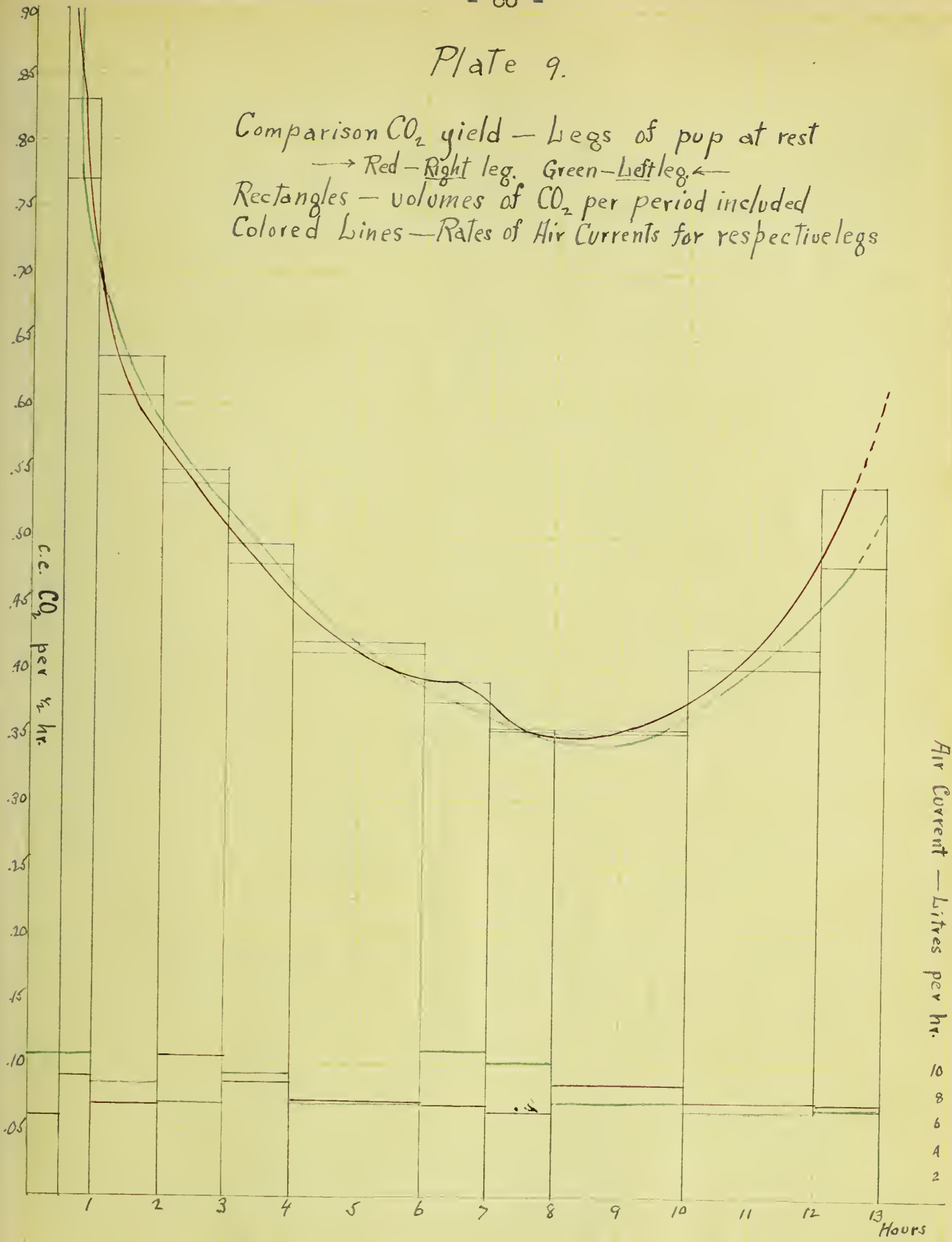On removal both legs were partly rigid, and had slight putrefactive smell.

Plate 9.

Comparison CO_2 yield — Legs of pup at rest
→ Red – Right leg. Green – Left leg ←
Rectangles — volumes of CO_2 per period included
Colored Lines — Rates of Air Currents for respective legs

Experiment 10

(a) The influence of contraction in CO_2 evolution by direct stimulation.

Method: Frog killed at 2:05 P.M., legs skinned, severed at pelvis and ankles.   Left leg placed in A, supported by wrapping electrodes around each ~~either~~ end of leg.   Right leg similarly arranged in B.   Both chambers closed at 2:43 and air currents allowed to run, unrecorded, until 2:55.   Ordinary coil used to produce induced current which passed to a metronome having adjustable time interval contacts, thence to electrodes on muscle. During period of contraction, muscle was given shock every 4 1/2 seconds for 5 minutes, then allowed to rest for 5 min., &c.

Each shock was slightly prolonged the fraction of a second through the slow action of the mercury contacts in the metronome.

Data:

Period	Length of Period	Conditions	Shocks given	Coil	CO_2 obtained Left	Right
1	65 min.	Left leg stim.	400	220-137mm	.354cc	.254cc
2	30 "	Both legs at rest			.120	.120
3	65 "	Right leg stim.	400	220-195	.210	.270
4	50 "	Both legs at rest			.160	.176

At end of experiment, both legs readily responded to direct stimulation, coil at 195 m.m.   It is to be noted that during contraction, both of the legs rubbed against wall of chamber.
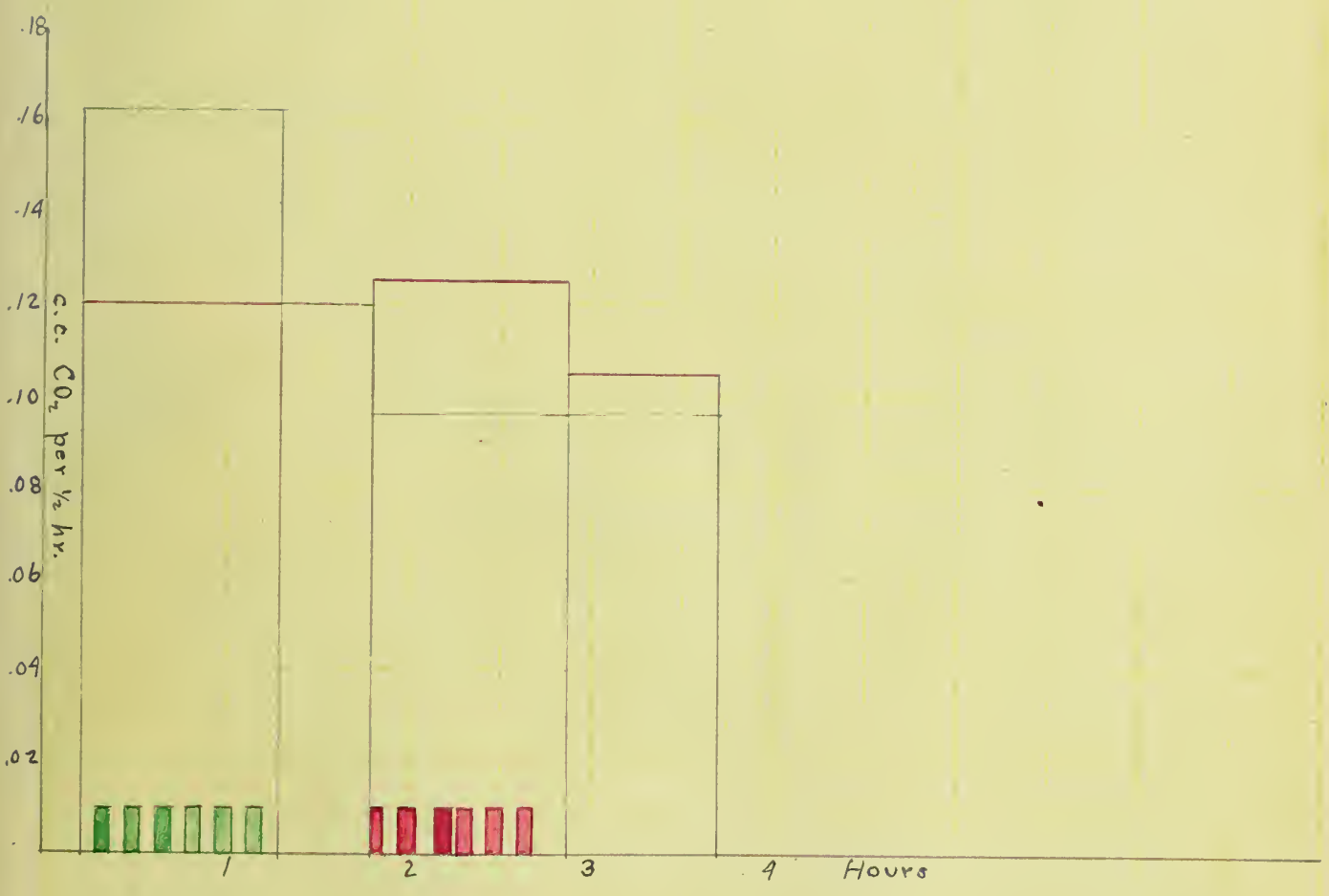
Plate 10.

Comparison CO_2 yield — frog's leg contracting vs. at rest.

Red: right leg.   Green: left leg.

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods.

Experiment 11.

The influence of contraction, by direct stimulation, on CO_2 evolution.

Method: (See Exp. 10)   Frog killed at 7:00 P.M.   Left leg in A, right in B at 7:10 P.M.   Current started at once but no record of yield until 7:25.   Stimulation every 4 seconds during each five minute period.   Temperature 23° C.

Data:

Period	Length of Period	Conditions	Shocks given	C o i l	CO_2 obtained Left	Right
1	1 Hour	Left leg stim.	450	185-168mm	.300cc	.214cc
2	1/2 "	Both legs at rest			.128	.128
3	1 "	Right leg stim.	450	200-180	.180	.260
4	1/2 "	Both legs at rest			.086	.099

At end of experiment, both legs readily responded to direct stimulation, coil at 180 m.m.   During contraction, both muscles rubbed against wall of chamber.
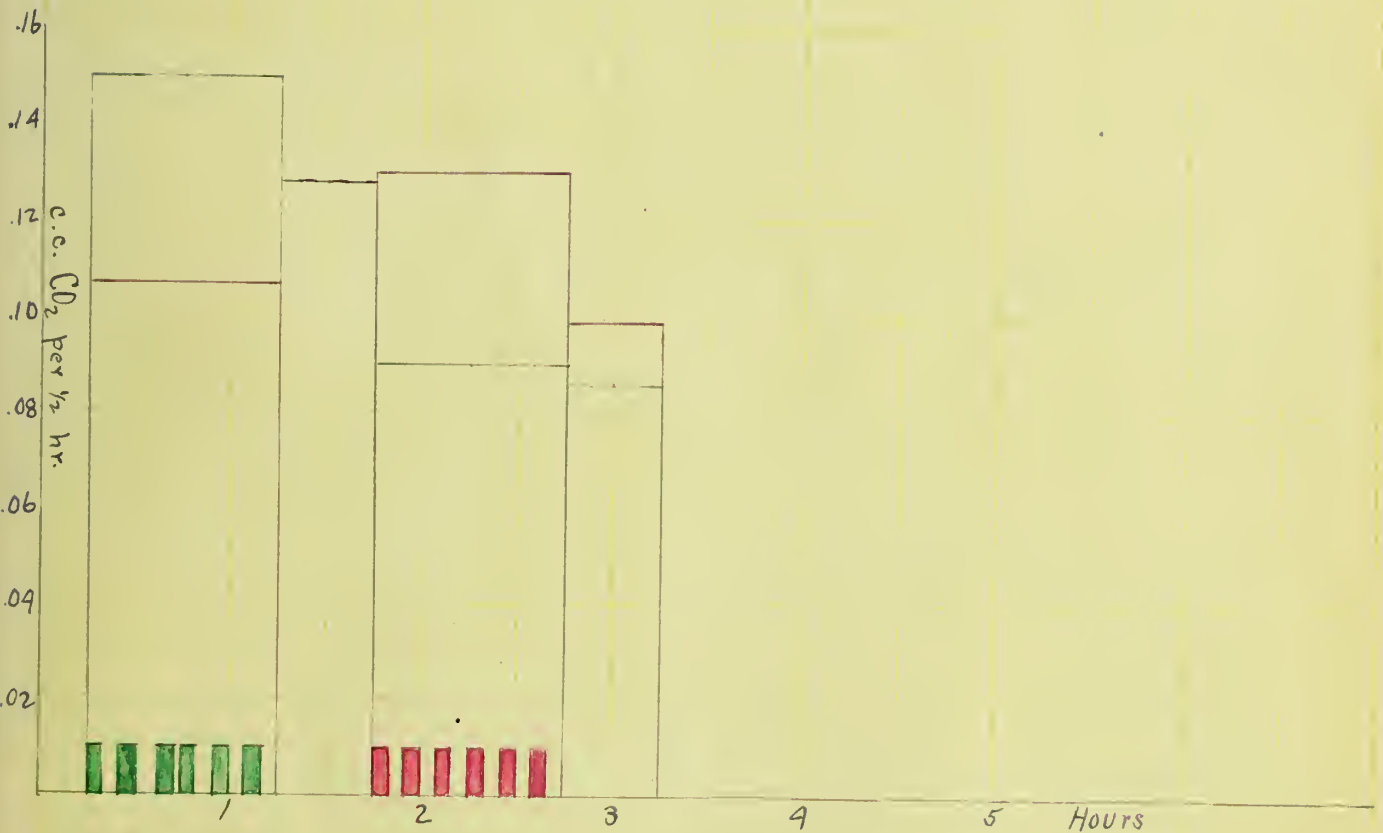
Plate 11.

Comparison CO_2 yield   frog's leg contracting vs. at rest.

Red : right leg.   Green : left leg

Rectangles — volumes of CO_2 per periods included.

Colored columns at foot — stimulation periods

Experiment 12.

The influence of contraction by direct stimulation on CO_2 evolution.

Method: (See Exp. 10)   Two frogs killed, 8:20 and 8:30 A. M. Used entire thighs with gastrocnemii attached.   The two right legs united at thigh ends by platinum wire and suspended upside down in A, with electrodes passed through each tendon Achilles.   Two left legs similarly arranged in B.   Shocks every four seconds, as ex- plained.   Temp. 22° C.

Data:

Period	Length of Period	Conditions	Shocks given	C o i l	CO_2 obtained Right	Left
1	30 min.	Both pairs of legs at rest			.198cc	.15cc
2	60 "	Right legs stim.	450	225-188mm	.236	.232
3	60 "	Both prs.of legs at rest			.176	.208
4	60 "	Left legs stim.	450	225-155	.180	.28
5	100 "	Both prs.of legs at rest			.252	.37
6	50 "	" " " " " "			.146	.19
7	60 "	" " " " " "			.17	.19
8	60 "	" " " " " "			.17	.20

At removal (3 hours after excision) both pairs of legs readily responded to direct stimulation, coil at 150 m.m.   During contraction, the left legs which hung in B rubbed against the wall of chamber.   The right legs in A hung freely.
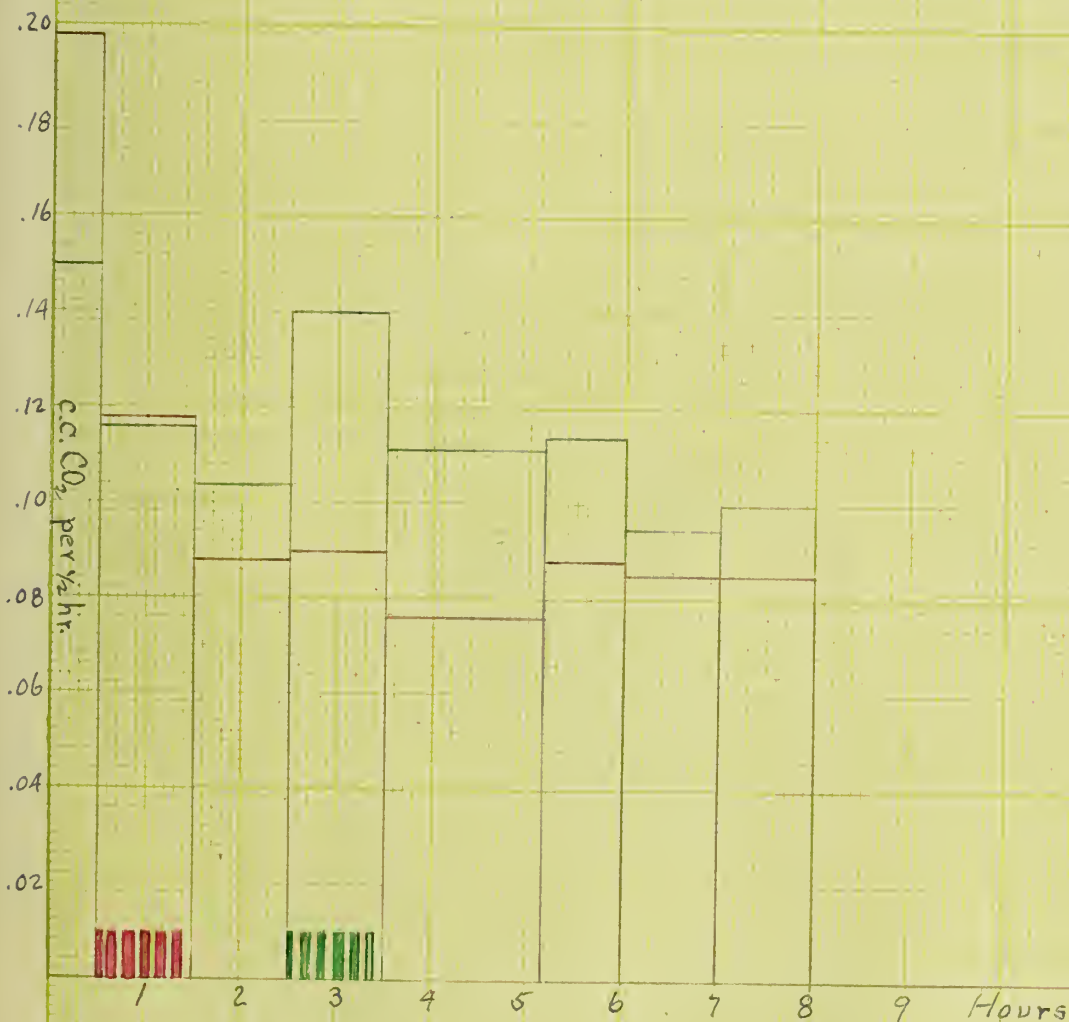
Plate 12

Comparison CO_2 yield — frogs' legs contracting vs. at rest.

Red: right legs.   Green: left legs.

Rectangles — volumes of CO_2 per period included

Colored Columns at foot — stimulation periods.

Experiment 13.

The influence of contraction by direct stimulation on CO_2 evolution.

Method: Two frogs killed at 7:15 P. M. Legs arranged as in Exp. 12. Air currents started at 8:18. No record until 8:35. Right legs in A, left in B. Shocks every four seconds (as explained in Exp. 10)

Data:

Period	Length of Period	Conditions	Shocks given	C o i l	CO_2 obtained Right	Left
1	30 min.	Both pairs of legs at rest			.08 cc	.112cc
2	55 "	Right legs stim.	450	225-150mm		
3	20 "	Both pairs of legs at rest			.274	.19

(Precaution was not taken to note whether the contracting legs rubbed against wall of chamber or not. It is very probable they did, because of their bulk and their arrangement similar to Exp. 12).
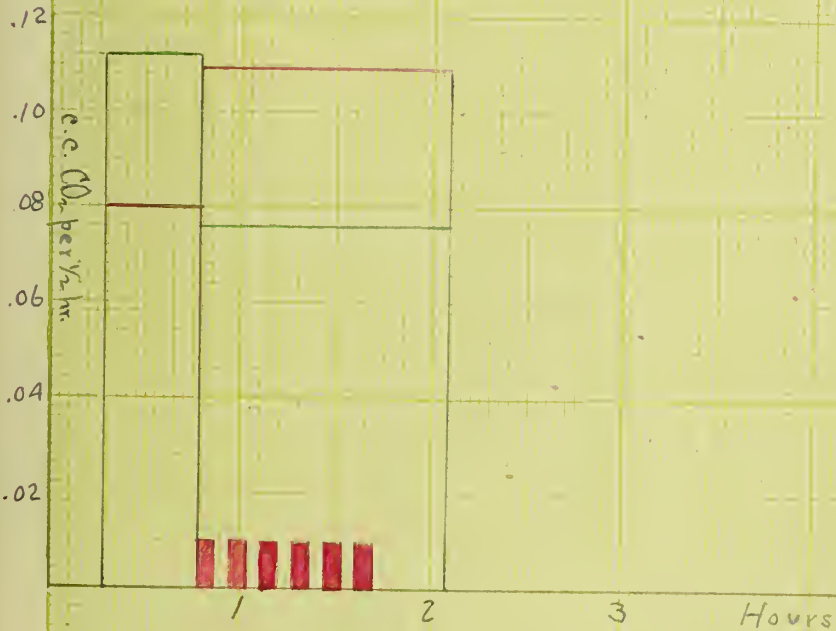
Plate 13

Comparison CO_2 yield — frogs' legs contracting vs. at rest

Red: right legs   Green: left leg

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

Experiment 14. *Ma 31*

The influence of contraction by direct stimulation on
on CO_2 evolution.

Method: (This can hardly be called a typical experi-
ment. A large bull frog had been killed by pithing both brain and
spiral cord 18 hrs. previously. The circulation to the left leg
was cut off by tying the left iliac artery, through a rather large
incision in the pelvis. An attempt was then made to curarize the
the frog by a hypodermic injection under the left shoulder, but
failed because of a partial loss of the curare through the large
opening in the pelvis and because of the sluggish circulation).
The gastrocnemii were both removed at 10:00 A.M. and left in .75%
NaCl until 11:00. That of the left leg, which had had no circu-
lation for 18 hrs., was placed in A. That of the right leg, cir-
culation slow but intact and also exposed to curare - providing the
drug had had any effect,-placed in B. Currents started at 11:00
A.M. No records until 12:16. Temp. 24-25° C. Shocks given as
explained in Exp. 10.

Data:

Period	Length of Period	Conditions	Shocks given	Coil	CO_2 obtained Left	Right
1	63 min.	Both muscles at rest			.14cc	.20cc
2	101 "	Left gastroc. stim.	517	165-50mm	.32	.38
3	77 "	Both muscles at rest			.16	.208

Upon removal, left gastrocnemius responded slightly by
direct stimulation, coil at 50 m.m. Right, very slightly, coil
at 0. (No record kept as to rubbing of contracting muscle on wall
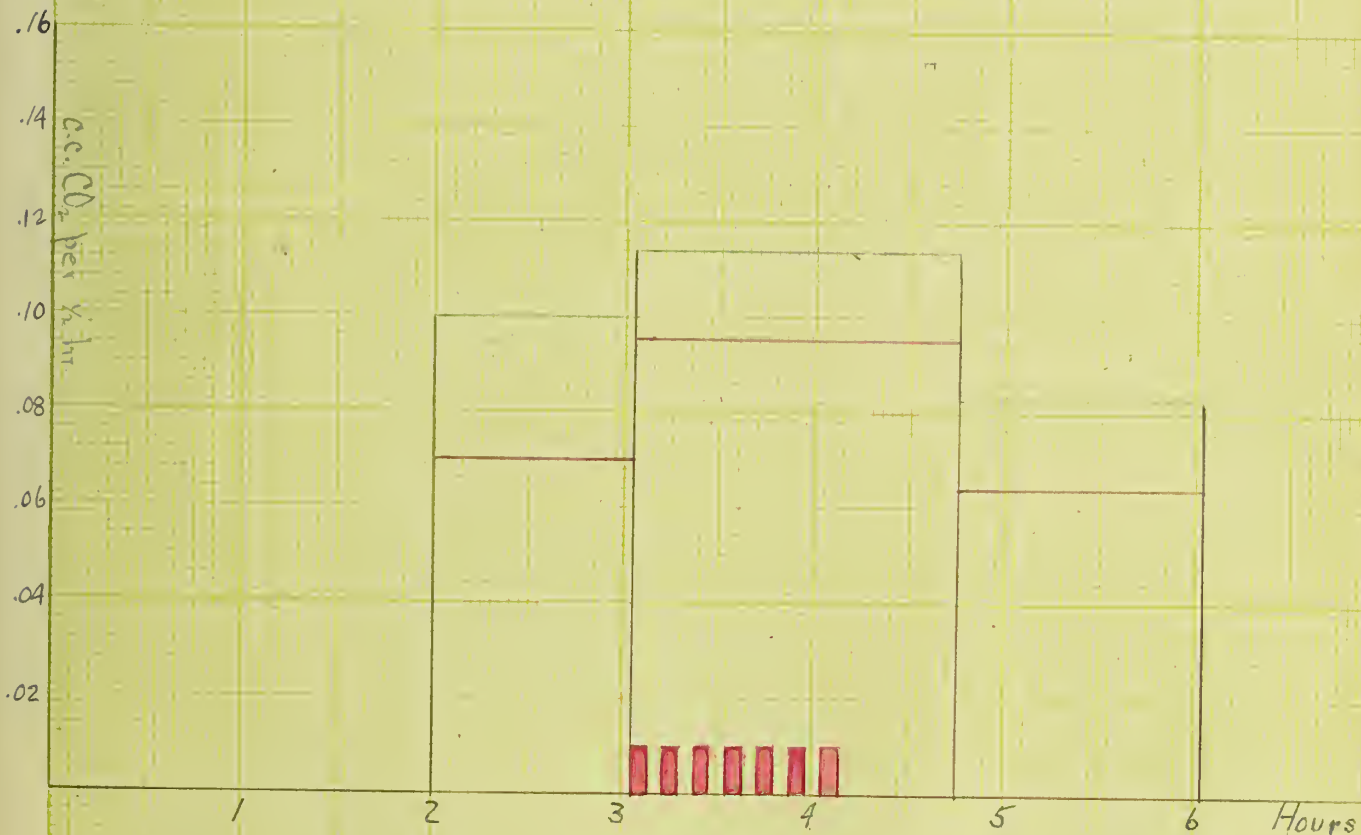of chamber).

Plate 14

Comparison CO_2 yield — gastrocnemius of large bull frog, previously

exposed to curare (see protocol), contracting vs. at rest.

Red: right gastroc.   Green: left gastroc. curarized.

Rectangles — volumes of CO_2 per period included.

Colored columns at foot — stimulation periods

Experiment 15.

Influence of contraction by direct stimulation on CO_2 evolution (pup).

Method. Nine weeks' old pup killed at 7:00 P.M. Right gastroc-nemius placed in A at 7:30, electrodes wrapped, one around muscles at head of former, the other around the tendon Achilles. Left similarly arranged in B at 7:50. Shocks given as explained in Exp. 10.

Data:

Period	Length of Period	Conditions	Shocks given	Coil	CO_2 obtained Right	Left
1	30 min.	Both muscles at rest			No record	
2	30 "	" " " "			.292 cc	.294cc
3	45 "	Right gastroc.stim.	225	100-60mm	.49	.334
4	30 "	Both muscles at rest			.22	.23
5	30 "	" " " "			.22	.20

Upon removal, neither muscle responded to direct stimu-lation. The muscles were of large size and during contraction, the right one rubbed against wall of chamber.
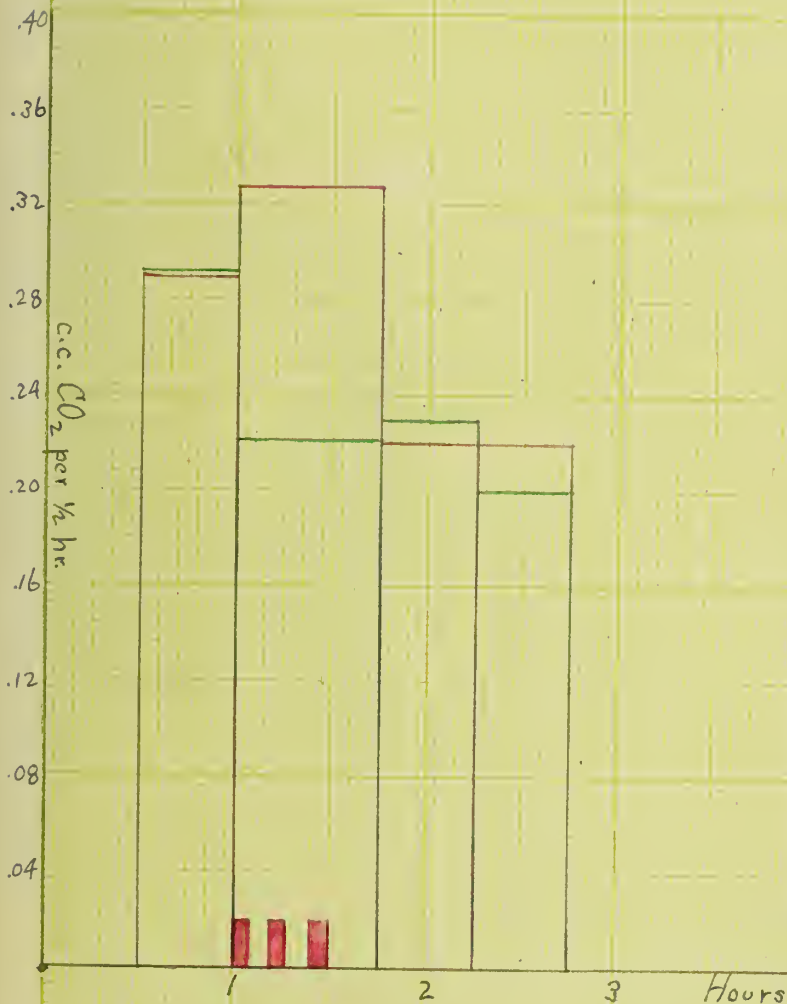
The image covers essentially the entire page as a scientific figure/plate.

Plate 15

Comparison CO_2 yield — pup's gastrocnemii, contracting vs. at rest

Red: right gastroc. Green: left gastroc.

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods.

Experiment 16.

(a) The influence of contraction by nerve stimulation on CO_2 evolution.

(b) The influence of contraction by direct stimulation on CO_2 evolution from curarized muscle.

Method: Dog, weight 7.5 kilo.,anaesthetized first by chloroform, then tracheotomized and ether substituted. Left gastrocnemius and nerve excised (2:40-3:05 P.M.) and hung by platinum wire in A; electrodes carefully coiled about inlet tube of chamber and the nerve thrown over these projecting ends. -- Dog was now curarized by injecting 1 c.c. of 1% solution of curare, dissolved in .60% NaCl, into jugular vein. It was the original intention to obtain a typical nerve stimulation experiment upon the curarized muscle, but, by mistake, the right sciatic nerve was severed down close to the gastrocnemius during excision. The muscle (somewhat lacerated) was placed in B at 3:18 for purpose of comparison and direct stimulation. Shocks as explained in exp. 10. Temp.28° C.

Data:

Period	Length of Period	Conditions	Shocks given	Coil	CO_2 obtained Left	Right
1	20 min.	Both muscles at rest			.254cc	.420cc
2	30 "	L.gastroc. stim.by nerve	150	300-100mm	.454	.494
3	20 "	Both muscles at rest			.266	.32
4	30 "	R.gastroc stim.directly	150	150-140mm	.43	.51
5	30 "	Both muscles at rest			.41	.454

Weight of L.gastroc after exp.was 21.3 gms. R. gastroc. 19.2 gms. (No record kept as to rubbing of contracting muscle on wall of chamber. Because of their large size,however,they probably did).
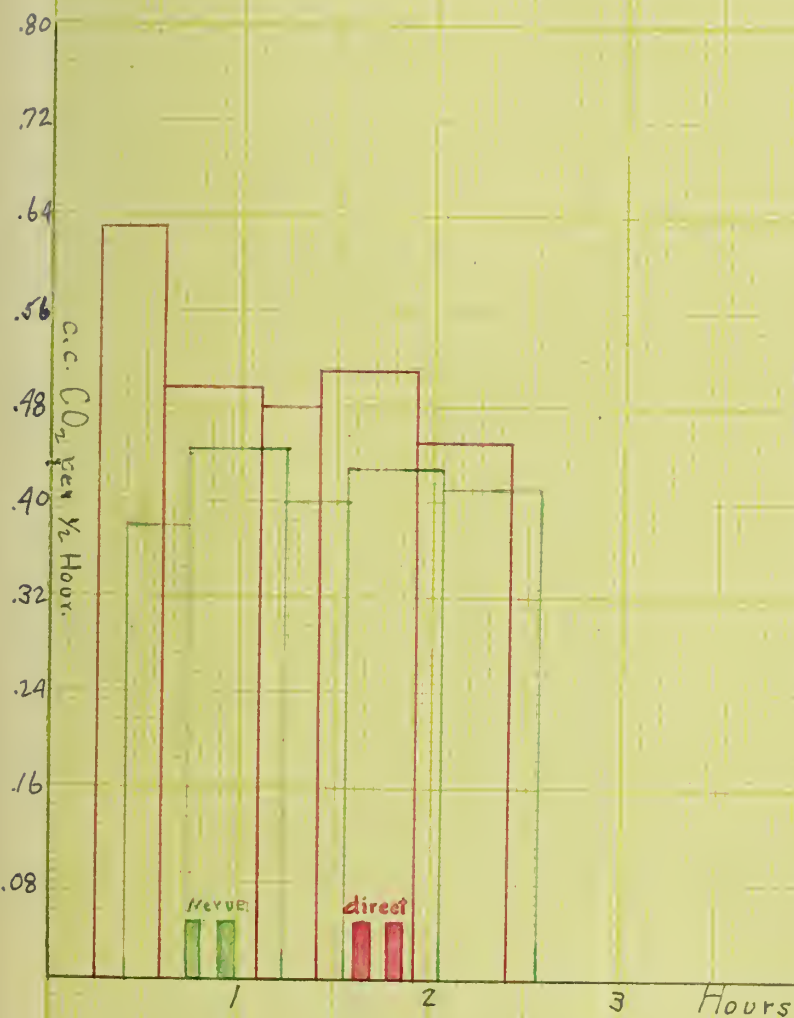
Plate 16

Comparison CO_2 yield — dog's gastrocnemii, contracting vs. at
rest (see protocol)

Red: right gastroc.   Green: left gast.

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

Experiment 17.

(a)Influence of contraction by nerve stimulation on CO_2 evolution.

(b)Influence of nerve stimulation on CO_2 evolution by curarized muscles.

Method: One frog killed, body severed through lumbar vertebrae, viscera removed, legs skinned, and feet amputated. Lumbar plexus of each side loosened and thrown over electrodes. Legs hung in B by platinum wire, coiled around urostyle, 3:35 P.M.

Another frog injected through dorsal lymph sac with 1/2 c.c. 1% curare in .60% NaCl, 12:00 M. After 3 hours, nerve stimulation failed to gain response, direct stimulation O.K. Legs prepared as above, and arranged in A. Shocks as explained in Exp. 10. Temp. 29° C.

Data:

Period	Length of Period	Conditions	Shocks given	Coil	CO_2 obtained Non-Cur.	Cur.
1	20 min.	Rest			.50 cc	.322cc[x]
2	40 "	Stim.both pairs of legs	225 ea.	330-140mm.	.87	.58+.17[t]
3	30 "	Rest			.62	.51
4	30 "	Rest			.44	.43

[x] No air current for ten minutes [t].58 c.c. during stimulation period $=$ first 30 min. .17 c.c. in rest period following $=$ 10 min. The curarized pair of legs were the larger. They were perfectly fresh when removed at 5:45 P.M. The non-curarized legs were stiff when removed. (No record kept as to rubbing of contracting legs against wall of chamber,but they probably did,if we may compare with similar conditions in Exp. 12).

Plate 17

Comparison CO_2 yield — frog's legs contracting vs. frog's legs curarized and nerves shocked.
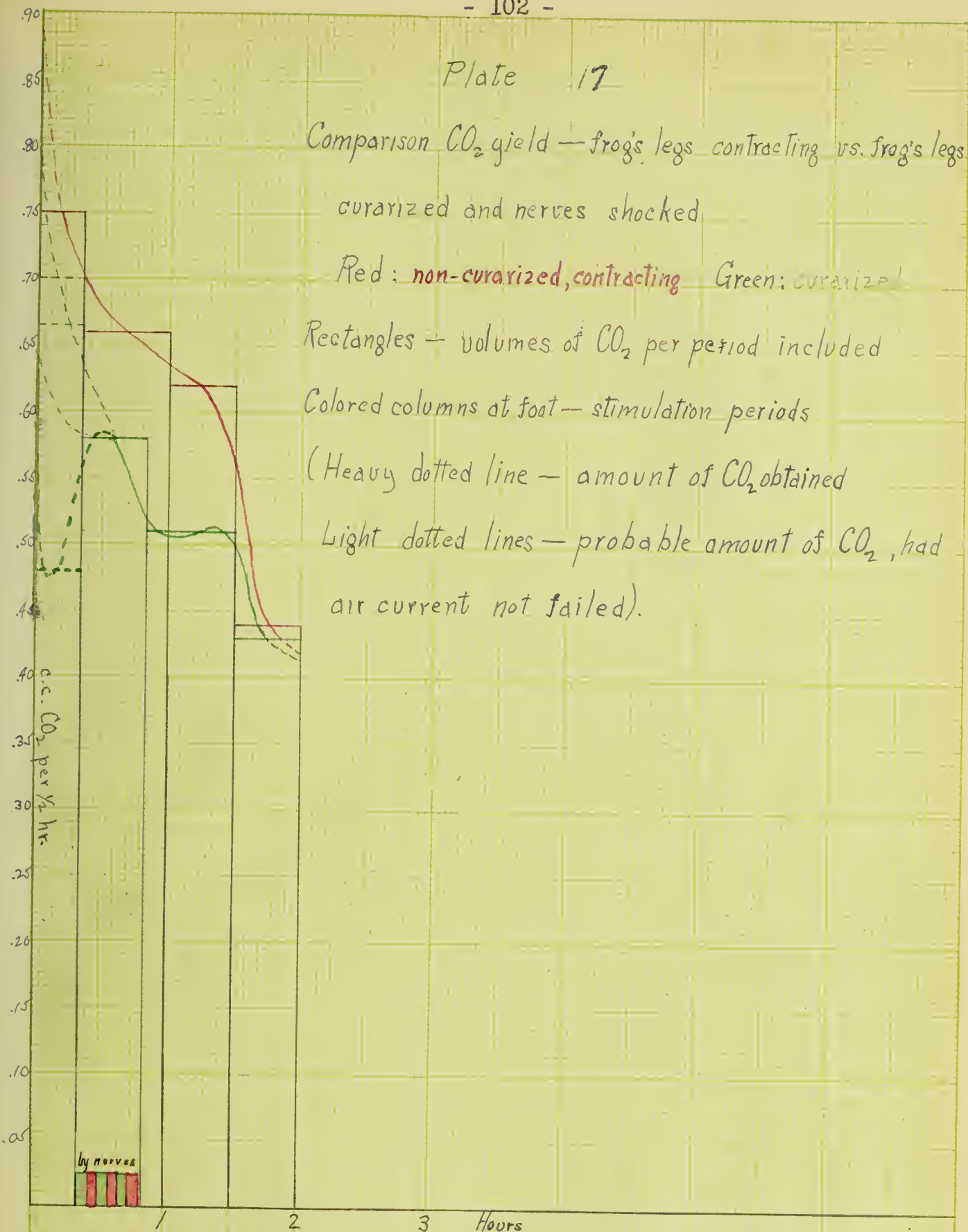
Red: non-curarized, contracting   Green: curarized

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

(Heavy dotted line — amount of CO_2 obtained

Light dotted lines — probable amount of CO_2, had air current not failed).

Experiment 18.

Influence of nerve stimulation on CO_2 evolution from curarized muscle.

Method: Frog curarized (6:00 P.M.) and legs prepared as in Exp. 17, 7:59.   Electrodes passed around lumbar plexus of each side so as to raise it up slightly from the dorsal body wall. The plexus not severed from that part of spinal cord still remaining in lumbar vertebrae.   Shocks given as explained in Exp. 10. Temp. 26° C.

Data:

Period	Length of Period	Conditions	Shocks given	C o i l	CO_2 obtained
1	30 min.	Rest			.456 cc
2	30 "	Stimulated by nerve 225		350-160 mm	.344
3	30 "	" " " 225		160-200 +	.286
4	30 "	Rest			.26
5	25 "	"			No record
6	30 "	Stimulated directly† 225		140-115	.32

+ During 2nd and 3rd periods, there was a tendency to spread of current from the short nerve attachments to the muscles of the back and thigh.   Whenever they contracted, the coil was at once raised.   † During the 6th period, the direct stimulation was by spread of current from short nerve attachment.

Upon removal of the legs at 11:00 P.M., nerve stimulation failed to give response.   A ready response to direct stimulation.   (No record kept as to rubbing of legs, during the contraction of the 6th period, on the chamber wall. As they were arranged similar to Exp. 12, they very probably did).
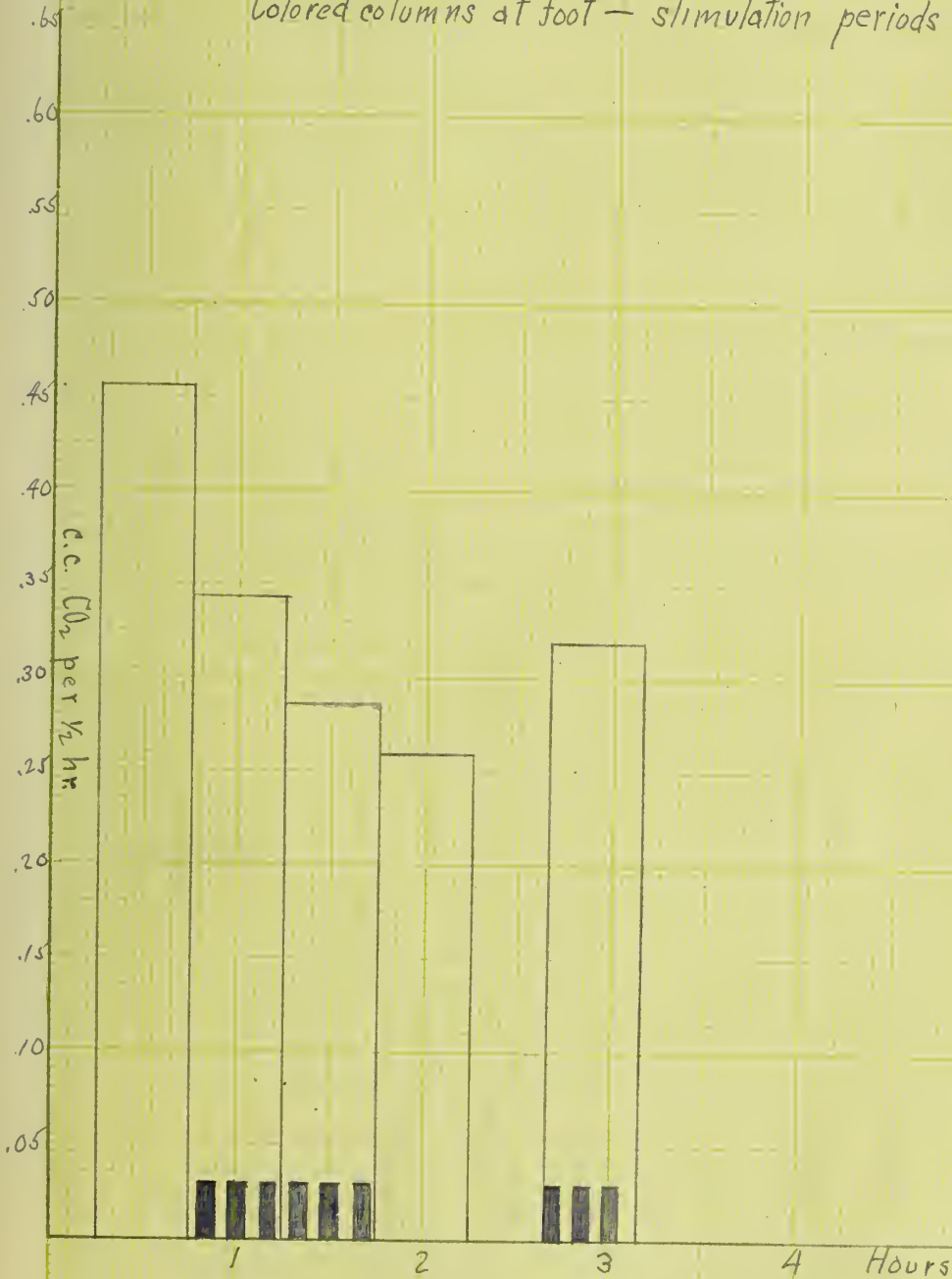
Plate 18

Curve of CO_2 yield from curarized frog's legs — nerves shocked; lastly, contraction by direct stimulation.

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

c.c. CO_2 per ½ hr.

Experiment 19.

(a) The influence of contraction on CO_2 evolution by nerve stimulation.
(b) The influence of stimulation by nerve on CO_2 evolution from curarized muscle.

Method: Cat, wt. 2.6 kilo., anaesthetized first by chloroform, then tracheotomized and ether substituted (see exp.16). L. gastrocnemius, with nerve, excised, and placed in A, electrodes on nerves. Wt. after exp., 23.5 grams.
Cat now curarized. Test--electrodes on nerve, no response. R. Gastrocnemius and nerve excised and placed in B. Wt. after experiment 22 1/2 grams.
The first period for A(all periods of 20 min. each)began at 3:55,-as close after excision as possible, that for B at 4:46. Shocks as explained in exp. 10. Temp. $30°-26°$C.

Data:

Period	Hours from start	Condition	CO2 obtained Non-curarized	Curarized
1	1/3 hrs.	Muscle at rest	.286 c.c.	.28 c.c.
2	2/3 "	" " stim. by nerve 150shocks,Coil 600-280 m.m.	.34 c.c.	.336c.c.
3	1 hrs.	Muscles at rest	.25 c.c.	.27 c.c.
4	1 1/3 "	" " " "	.28 c.c.	.28 "
5	1 2/3 "	" " " "	.26 " 5-8Per	--------
6-10	3 1/3 "	" " " "	--- 9th "	.246c.c.
11	3 2/3 "	" " " "	.29 "10-11"	-------
12-13	4 1/3 "	" " " "	--- 12"	.236c.c.
14	4 2/3 "	" " " "	.30 13-14"	-------
15-16	5 1/3 "	" " " "	--- 15"	.236c.c.
17	5 2/3 "	" " " "	.326cc.16-17"	-------
18-19	6 1/3 "	" " " "	--- 18"	.232c.c.
20	6 2/3 "	" " " "	.354	-----------

Muscles removed; left one slightly stiffer than the right. During the first periods, surface of both muscles became very red due to exudation of blood. No record kept as to "work" performed during contraction, but as the muscles were large, the contracting one undoubtedly rubbed on walls of chamber.
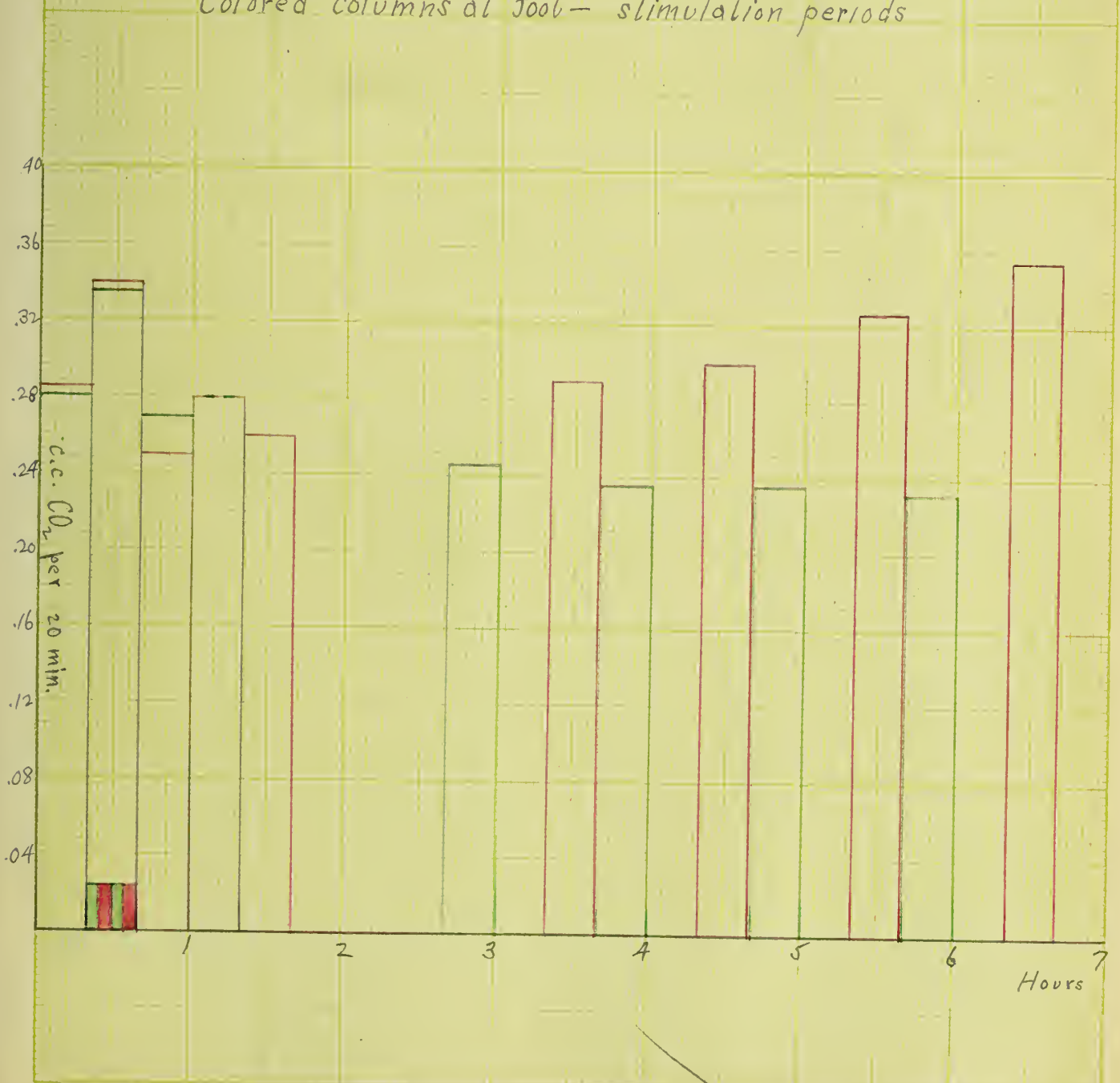
Plate   19

Comparison CO_2 yield — Cat's gastrocnemius, curarized, shocked by

nerve vs. ditto non-curarized, contraction by nerve stimulation

Red: non-curarized.   Green: curarized.

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

Experiment 20.

(a) The influence of contraction on CO_2 evolution by nerve stimulation.

(b) The influence of stimulation by nerve on CO_2 evolution from curarized muscle.

Method: Large frog, killed by pithing brain, at 2:10. R. gastroc. and nerve excised at 2:25 and placed in A. Frog then curarized by opening thorax and injecting 1/2 c.c. of 1% curare sol. (in 75% salt sol.) into apex of ventricle. Drug took effect very rapidly; test of action - pinched bachial nerve - no response. L. gastroc then excised and placed in B. Shocked as explained in exp. 10. 1st Per. for A started at 2:35; for B, at 3:05. All periods 1/2 hr. long. Temp. 34-31°C.

Data:

Period	Length of Period	Conditions	CO_2 obtained Non-cur.	Curarized
1	30 min.	Muscles at rest	.26 c.c.	.26 c.c.
2	30 "	Muscles stim. by nerves - 225 shocks - coil 450-90 m.m.	.26	.284 "
3	30 "	Muscles at rest	.24	.286 "
4	30 "	Muscles at rest	.24	.27 "

Right muscle removed at 5:00 - had perceptibly shortened, but was not stiff. Here no record was kept of possible work performed. No response to direct stimulation. Wt. on removal, 7.2 grms.

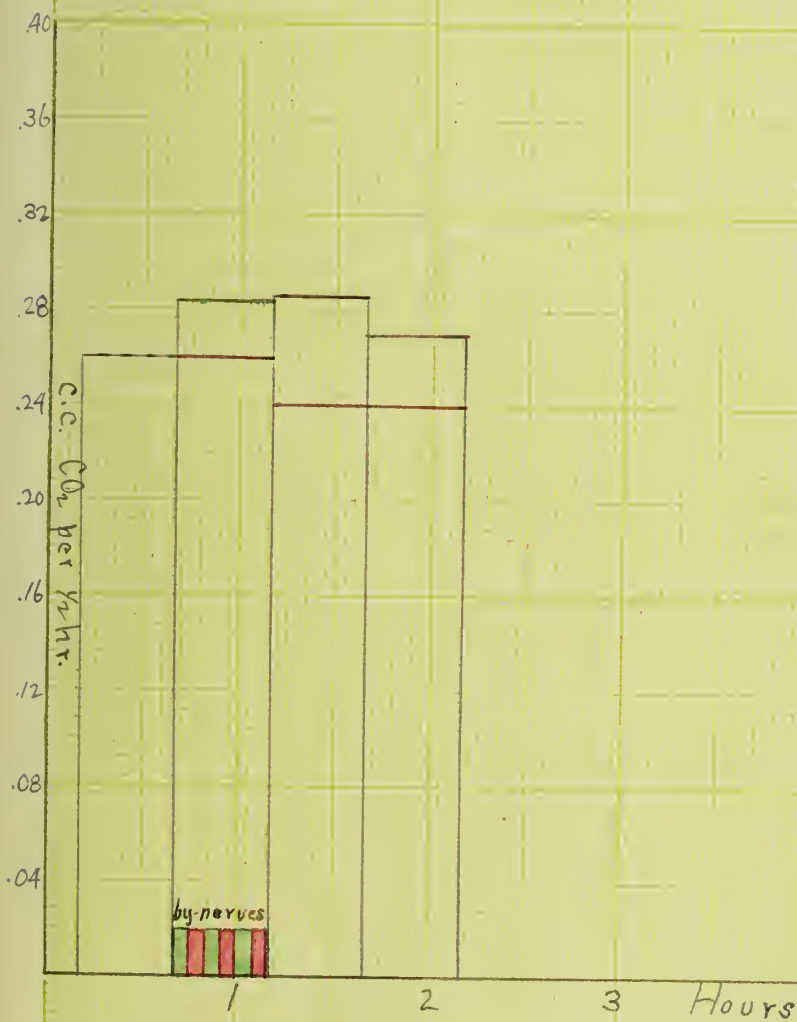Left muscle removed at 5:05. Responded to direct stim., coil at 70; wt. on removal 7.4 grms.

Plate 20

Comparison CO_2 yield — gastrocnemius of frog, curarized, nerve shocked vs. ditto, non-curarized, contraction by nerve stimulation

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

Red: **non-curarized, contracting.** Green: curarized

Experiment 21.

To determine amount and rate of CO_2 yield from frogs' muscles at rest.

Method: Large bull-frog killed at 8:13 A. M.   R. gastrocnemius excised at 8:25, and put in A at 8:29.   Wt. on removal 5.6 grams.   L. gastrocnemius excised at 8:38, placed in B at 8:42. Wt. on removal, 5.5 grams.   Electrodes arranged for direct stimulation at any time.

Data:

| Hours from start	CO_2 obtained Right	Left	CO_2 rate pr 1/2 h. Right	Left	Temp.	Remarks
1/3	.16cc	.14cc	.23	.20	26° C	
2/3	.14	.13				
1	.09	.10	.16	.17		
2	.36	.34	.18	.17	28	Direct stim.,both muscles responded.
3	.31	.31	.16	.16	30	Direct stim.,both muscles responded.
4	.31	.30	.16	.15	31	Direct stim.,both muscles responded.
5	.29	.28	.15	.14	31	" "
6	.28	.26	.14	.13	30	" "
7	.24	.24	.12	.12	30	" "
8	.23	.23	.12	.12	30	" "
9	.22	.18	.11	.09	30	Right leg failed to respond
10	.26	.20	.13	.10	30	Left leg failed to respond
11	.28	.24	.14	.12	30	

Both muscles when removed at 8:10 P. M. were stiff, opaque and shortened.   The response to stimulation was tried at end of each hour, starting in 2nd hour with coil at 160 (for Right) - 210 (for Left) m m. and ending with zero at end of 9th and 10th hours.

Plate 21

CO_2 yield — gastrocnemius large bull frog at rest vs. ditto.

Red: *right* gastrocnemius. Green: *left* gastrocnemius.

Rectangles — Volumes of CO_2 per period included
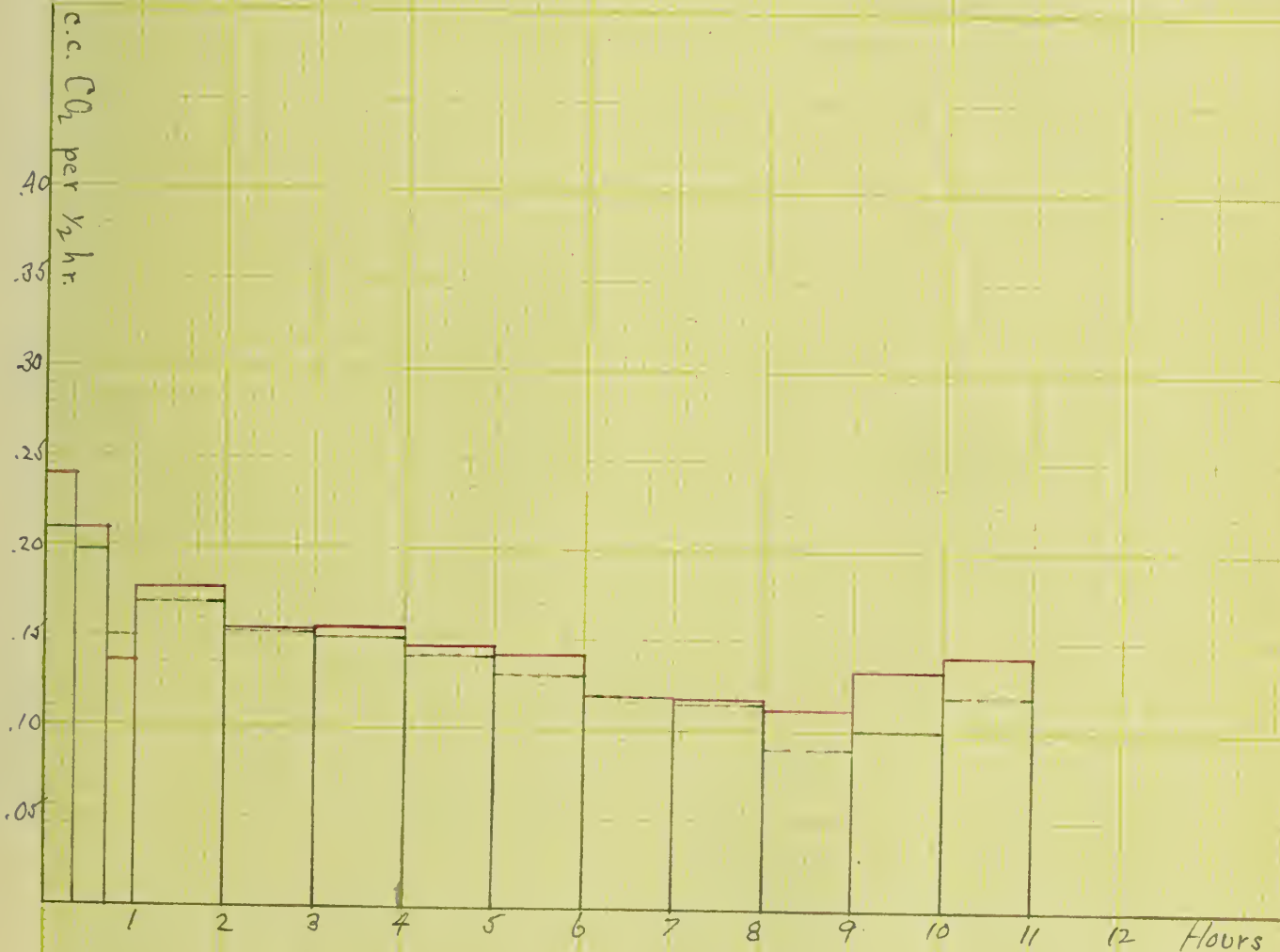
Experiment 22

Influence of contraction by nerve stimulation on CO_2 evolution.

Method: A large bull frog, in the last stages of the disease "red leg", pithed at 9:12 P. M. R. gastrocnemius excised at 9:20, put in A and current started at 9:34, electrode on nerve. Shocked as explained in exp. 10, temp. 30° C.

Data:

Period	Length of Period	Condition	Shocks	Coil	CO_2 obtained
1	1/2 hr.	Rest			.288 c.c.
2	1/2 "	Stimulated by nerve	225	390-305mm	.211
3	1/2 "	Rest			.14
4	1/2 "	"			.16

Experiment discontinued because stimulation in this nerve had suddenly ceased at beginning of 4th period. At end, muscle responded by direct stimulation with coil at 200 mm. This muscle hung loose and free.
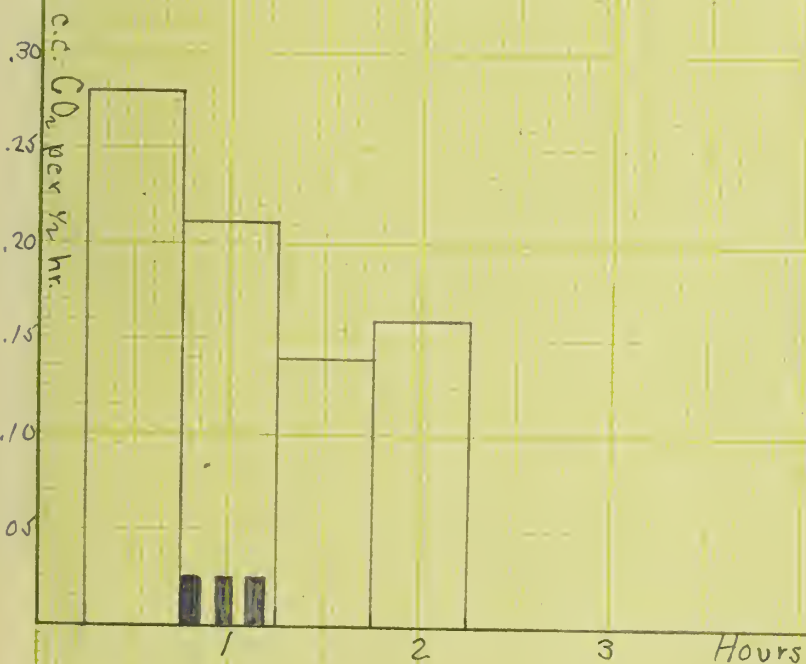
Plate   22

CO_2 yield — gastrocnemius large bull frog, contracting by nerve

stimulation

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

Experiment 23.

Influence of contraction by nerve on CO_2 evolution.

Method. A large bull frog, suffering with "red leg", pithed at 1:55 A. M. R. gastrocnemius excised at 2:00, put in A and current started at 2:13, electrodes on nerve. Shocked as explained in Exp. 10. Temp. 28° C.

Data:

Period	Length of Period	Condition	Shocks	C o i l	CO_2 obtained
1	1/2 hr.	Rest			.306 c c
2	1/2 hr.	Stimulated	75	755-720mm	.25
3	1/2 hr.	Rest			.17

Exp. discontinued because the muscle failed to respond to nerve stimulation after the first 5 min. During the 5 min. the muscle responded, it was the most sensitive yet found. (Notice height of coil compared with other exp.) Just before removal muscle responded all right to direct stimulation. The muscle hung loose and free.

Plate   23

CO_2 yield — gastrocnemius of large bull frog, contracting by

nerve stimulation

Rectangles — volumes of CO_2 per period included

Shaded area — one period of stimulation

Experiment 24.

(a) Influence of contraction on CO_2 evolution by nerve stimulation.
(b) Influence of stimulation by nerve on CO_2 evol. from curarized muscle

Method: Large bull frog, suffering with "red leg", pithed at 4:08 A.M. R.gastroc.excised 4:10, put in A at 4:28, electrodes on nerve. Wt. After exp.,6 grams. Frog curarized through heart (see exp. 20) 4:35. Test,-elec.stim.of brachial nerve gave no response 4:50. L. gastroc.excised 4:55, put in B, 5:05, electrodes on nerve. Wt. after exp. 6.3 grams. Shocked as explained in exp. 10.

Data:

Period	Hours from start	Conditions	CO_2 evolved (R.non-cur.)	(L.-cur.)	Temp.
1	1/2	Both muscles at rest	.21cc	.29cc	28°C
2	1	Stim.both muscles by nerves 225 shocks,coil 600-540 m.m.	.14	.21	26°
3	1 1/2	Stim.both muscles by nerves 225 shocks,coil 600-540 m.m.	.15	.17	
4	2	Both muscles at rest	.11	.16	
5	2 1/2	Both muscles at rest-no record-current stopped 18 min., then pumped out vigorously 12 min.			
6	3	Both muscles at rest	.13	.11	24°
7	3 1/2	Stim.both muscles by nerves 225 shocks,coil 538-523 m.m.	.10	.10	
8	4	R.muscle at rest;by mistake stim. L.5 min.	.10	.11	
9	4 1/2	Stim.both muscles by nerves 225 shocks,coil 415-170 m.m.	.09	.15	
10	5	Both muscles at rest	.10	.14	27°

At 5 1/2 hrs.,R.gastroc.readily responded to nerve stim. Coil at 170 m.m. Muscle was quite red in appearance.

At 11 hrs.,both muscles readily responded to direct stim. Coil at 115 m.m. At 12 1/2 and 12 hrs.,all the gas which had collected since the 5th hr.from the muscles remaining in chambers (no current) was passed over and recorded. The R.gastroc.had been under slightly positive pressure for 7 hrs.previous. At end of 7 1/2 hrs.,yielded for the total period 1.51cc. CO_2. L.gastroc.had been under slightly negative pressure for 6 1/2 hrs.previous. At end of 7 hrs.,yielded for the total period, 1.24cc CO_2.Temp. 28°.

At 14 hrs.,R.gastroc.yielded for 2 hr.period previous, current going, 1.23cc CO_2. Still responded to direct stim. previous.
At 13 1/2 hrs., L.gastroc.yielded for 2 hr.period, current going, .73cc.CO_2. Slight quiver to direct stim.
At 14 hrs., R.gastroc.removed; not rigid, natural color.
At 13 1/2 hrs., L.gastroc.removed, partly rigid, natural color.

These muscles retained their irritability the longest of any muscles experimented with. The R. muscle during its periods of contraction hung free from contact with the walls.
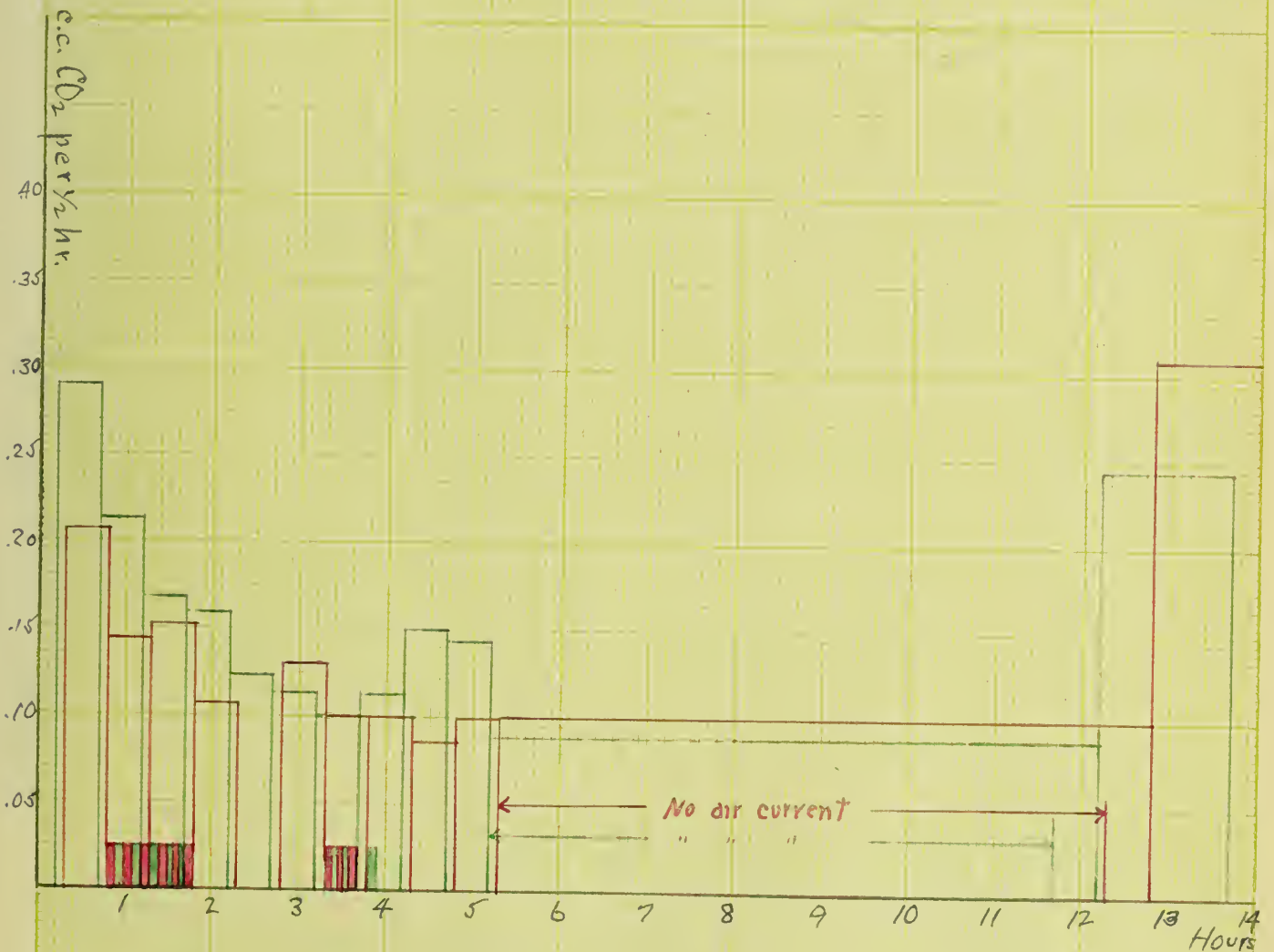
Plate 24

Comparison CO₂ yield – gastrocnemius large bull frog, stimulated to contraction by nerve vs. ditto curarized, nerve shocked

Red: non-curarized, contracting. Green: curarized.

Rectangles — volumes of CO₂ per period included

Colored columns at foot — stimulation periods

Experiment 25.

(a) Influence of contraction by nerve and by direct stimulation on CO_2 evolution.

(b) homologous muscle at rest.

Method: Large bull frog, suffering with "red leg", pithed at 4:58 P.M. R. gastroc., excised 5:00, put in A at 5:15. Wt. of muscle after exp. 4.7 grams. On trial, responded to nerve stim., coil at 400 m.m. L. gastroc. excised 5:15, put in B at 5:22. Wt. of muscle after exp. 4.9 grams. On trial, responded to nerve stim., coil at 525 m.m. Electrodes on nerves in both chambers. Shocks as explained in Exp. 10. Temp. 29° C.

Data:

Period	Length of Period	Conditions	Shocks	C o i l	CO_2 obtained Right	Left
1	30 min.	Rest			.225cc	.28cc
2	60 "	Stim.L.gastroc.by nerve	450	550-445mm	.35	.42
3	30 "	Rest+			.13	.15
4	60 "	"			.256	.264
5	60 "	Stim.L.gastroc directly	390	165-145mm	.23	.25
6	60 "	Rest			.22	.23

+ At beginning of 3rd per., R. gastroc. would not respond to nerve stim ,although it would to direct; coil at 155 m m. Exp. discontinued, R. muscle would no longer give response to direct stim.; natural color, not stiff. L. muscle readily responded to direct stim., coil at 140 m.m.; natural color, not stiff. In this experiment, the L. gastroc. during contraction hung free from wall of chamber.
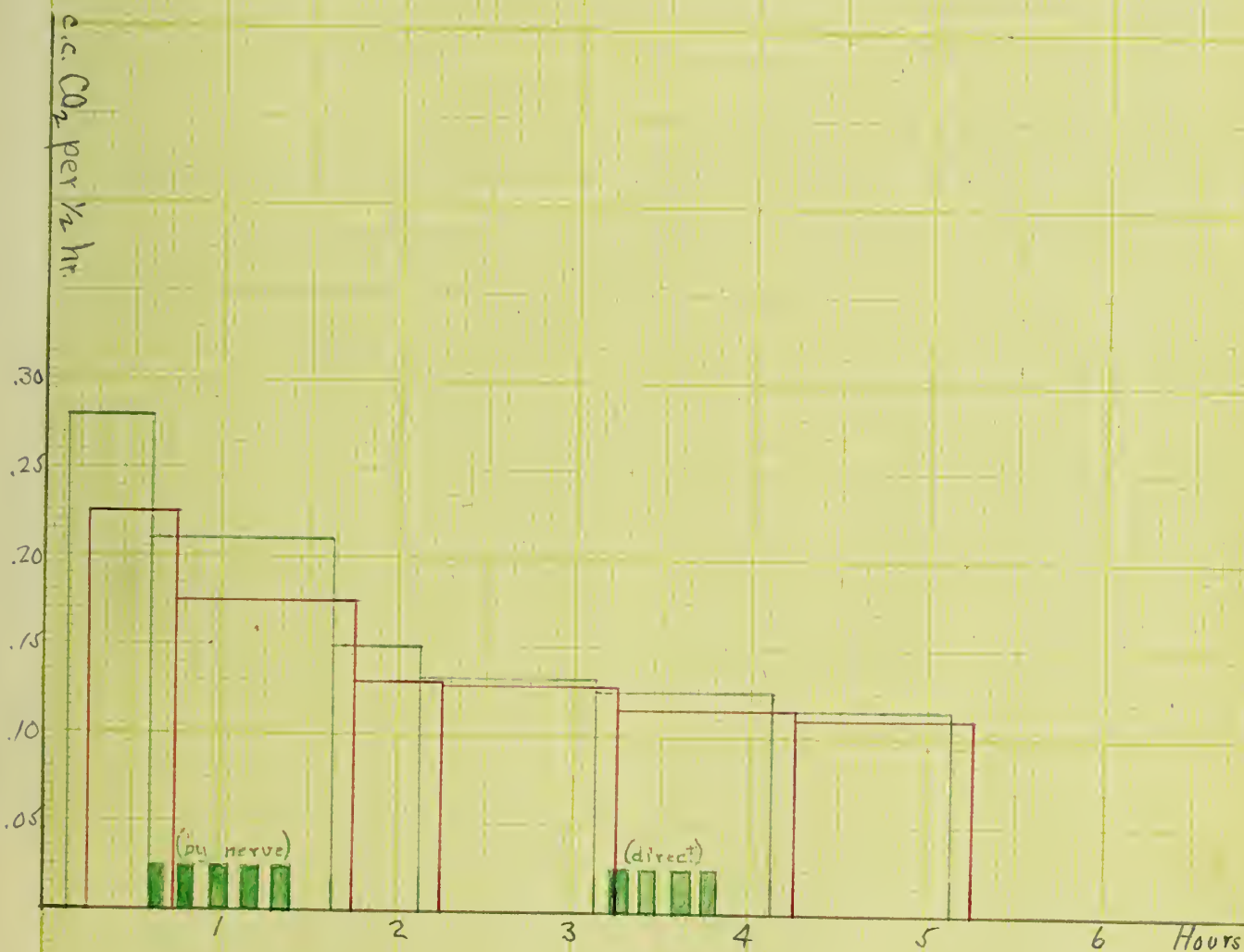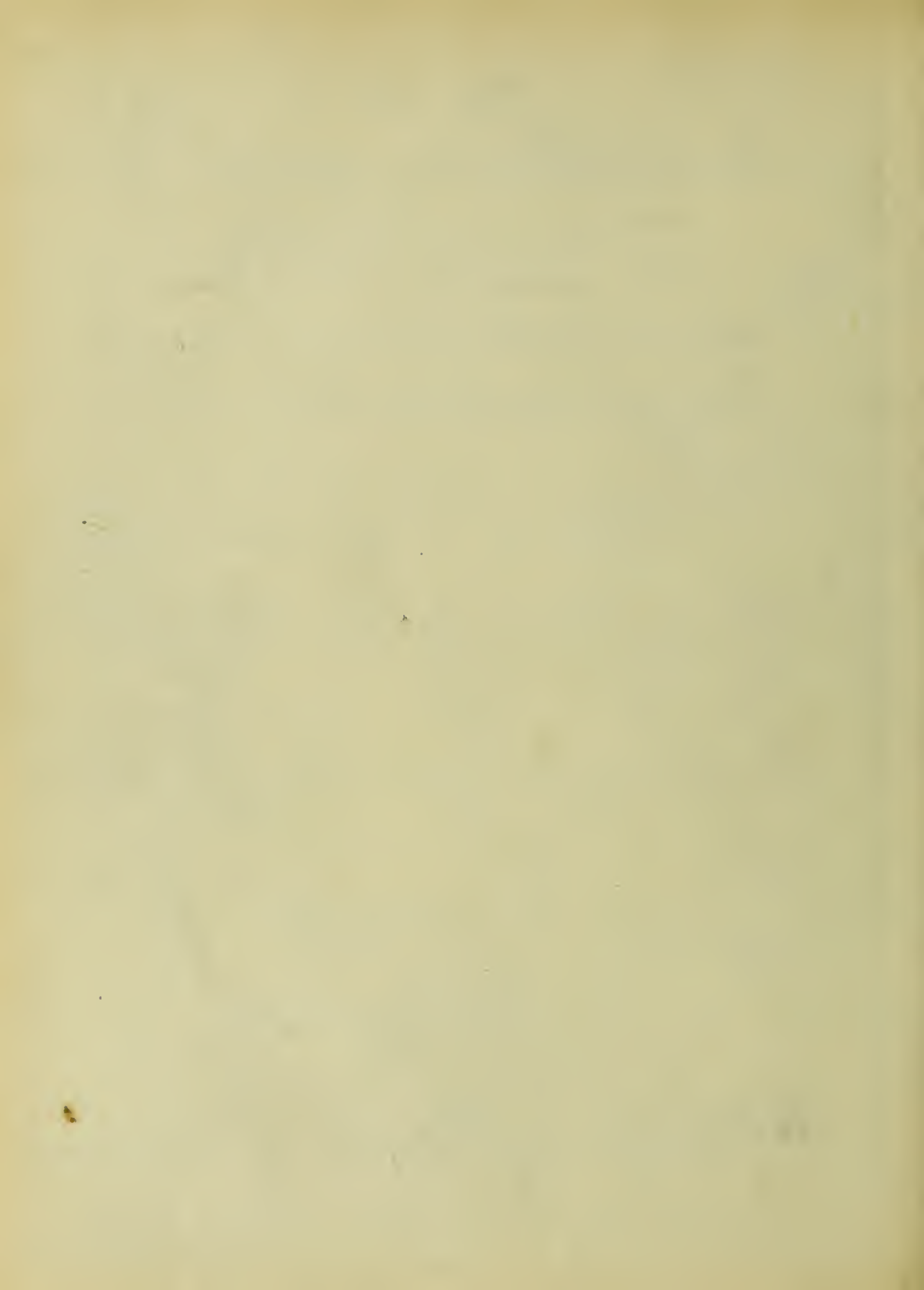
Plate 25

Comparison CO_2 yield — gastrocnemius large bull frog at rest vs. ditto

contracting (1) by nerve (2) by direct stimulation

Red: **muscle at rest.**   Green: contracting muscle.

Rectangles — volumes of CO_2 per period included

Colored columns at foot — stimulation periods

Summary of Improvements in Apparatus

1.   The apparatus here described possesses all of the advantages of that used by Blackman, by Fletcher, and by Stanley (see pages 7 - 11).

2.   In addition to the above it possesses the following advantages and modifications introduced by us:

(a)   A new method of absorption by double Winkler spirals.

(b)   The employment of the double spiral makes it possible to use weaker standard solution, thereby increasing the delicacy of the determination.

(c)   The possibility of employing a faster rate of air current than could be used in any apparatus previously described.

(d)   By means of this faster air current, to remove the CO_2 more promptly from around the muscle.

(e)   By means of this faster air current, to divide the experiment into shorter periods, if so desired.

(f)   To obtain a higher degree of accuracy in the estimation of the CO_2 than has hitherto been found for any apparatus employing a constant current.

Summary of Findings of Chemical Interest.

1. On account of the great solubility of CO_2 in weak acid solution and the unsteady CO_2 equilibrium in the same, all such solutions, intended to be used for titration in the cold with phenolphthalein, even though in closed systems away from the influence of atmospheric CO_2, must be made of CO_2 free water and carefully preserved from contact with the air.

2. On account of changes in the end reaction when titrating baryta against standard mineral acid in the presence of $BaCO_3$-precipitate and agitation, all alkaline salt -- sodium or potassium-must be absent. (See page 123). Therefore sodium and potassium salts must be excluded from both HCl and baryta standard solutions to be used in estimating CO_2 .

3. $BaCO_3$ precipitate when lodged upon the walls of the chamber, has a great absorptive power for neutral or alkaline solutions which may come in contact with it. Consequently a weak baryta solution may sustain a considerable loss in this manner which might otherwise be attributed to CO_2 absorption.

4. Definite amounts of CO_2 may be freed from measured samples of a solution of Na_2CO_3 by dropping the latter into strong H_2SO_4 and passing a brisk CO_2-free air current through the same. We have found it necessary to use a special apparatus for this. (Page 50).

Summary of Findings of Physiological Interest.

1. Normal isolated muscles at rest, whether frog or mammalian, produce a constant evolution of CO_2 , greatest in amount immediately after excision, but gradually decreasing, with some

irregularities, for many hours thereafter.

2.   The greatest irregularities are noted in frog's muscle, which, about the third hour after excision, shows either a check in the rapid decline of the yield or a temporary increase. Again, at the sixth or seventh hour, there may be a marked increase or an equally noticeable decline.   After the seventh hour the yield becomes nearly steady for hours, or perhaps gently decreasing, until putrefactive processes begin when there is a sudden increase in the yield.

3.   With mammalian muscle, the decline in the rate of CO_2 yield gradually continues for ten hours at least, and with scarcely any irregularities in the yield whatever.

4.   Contracting muscles, which are not fatigued, may or may not show an increased yield of CO_2 .   We have considerable evidence to show that where work is done there is an increased yield of CO_2 by the muscle.   Otherwise contraction seems to have no influence upon the amount of the CO_2 produced.

5.   There appears to be no difference in the CO_2 yield from a muscle stimulated through the nerve versus one stimulated by applying the electrode directly to the muscle.

6.   Variations in the ordinary room temperature (18 - 31°C.) appear to have no material influence on the normal rate of yield. The greatest variation in any one experiment was five degrees centigrade.

7.   Variations in the speed of the air current, or in the negative pressure, (within the limits given on page 63), used to remove the CO_2 from the muscle, have no influence upon the rate of yield.

8.   A total suppression of the air current produces a sudden   fall *in* ~~of~~ the rate of the CO_2-yield.

9.   Curare does not appear to have any influence upon the regular rate of CO_2 yield from frog's muscle unstimulated.

10.   Curarized frogs' muscles, when their nerves are stimulated have not given constant results so that further work in this field will be necessary before drawing definite conclusions.

11.   Curarized mammalian muscles, when their nerves are stimulated yield a larger amount of CO_2 than when remaining unmolested.   This statement is based upon one experiment, whence further work in this field is also necessary.

APPENDIX

Note 1 -- All flasks and bottles used for standard solutions were cleaned by strong acid, tap water, and distilled water.

Note 2 -- To dry the supply bottles, each is fitted with a two-holed cork having one glass tube long enough to reach to the bottom of the bottle and another ending flush with the inside of the cork. The bottle is inverted and the short tube attached to the Bunsen pump.   The long tube is attached to a large Winkler spiral containing con. H_2SO_4 and this in turn to a similar spiral filled with strong potash.   The current is then started and the air, freed of atmospheric CO_2 and dried, rapidly dries out the bottle and replaces the contained atmospheric air.   We have found this mode of procedure imperative, for the rinsings left in the carefully drained bottle, even when CO_2 -free water is used, absorb CO_2 from the air to such an extent that they will cause a marked precipitate of $BaCO_3$, should the bottle be filled with $Ba(OH)_2$.   Furthermore, the bottle at first contains enough atmospheric CO_2 to cause the same effect.

Note 3 -- We have found that no appreciable amount of CO_2 is absorbed from the atmosphere by the "luke-warm" water while pouring from one bottle to another.   All undue exposure must, of course, be avoided.   The bottles and flasks should be corked at once after the transfer.

Note 4 -- We made a large number of experiments with different samples of Barium Hydrate, and found that if the least trace of Sodium or Potassium could be demonstrated in the sample by the platinum wire flame test, the sample must be discarded.   The reason for this is that the presence of either of these metals in the baryta solution which has been partially precipitated as $BaCO_3$ causes a

rapid re-appearance of color; i.e., return to alkalinity, after ti-
trating the excess $Ba(OH)_2$ to neutrality with phenolphthalein. This
color re-appearance was found to depend upon the amount of contami-
nating alkali present, the amount of $BaCO_3$ precipitate formed,and
upon the speed of the air bubbles used for stirring up the solution
during titration. The following experiment will illustrate:--20c.c.
of an N/100 $Ba(OH)_2$ solution, containing a trace of alkali,was first
titrated in the absorption chamber with HCl and phenolphthalein,and
a neutral point obtained which remained for an hour or more in the
presence of the most vigorous bubble-stirring. Again a like amount
of the same baryta solution was taken and CO_2 (from the room air)
passed through it until an easily visible precipitate of $BaCO_3$ was
formed. If the excess $Ba(OH)_2$ was now just neutralized with HCl,
it was found that the color re-appeared; i.e., alkali was re-formed,
almost immediately in the presence of the stirring,and kept steadily
increasing in amount. In fact, it was found impossible by a stream of CO_2, to remove
all the color in a stirred solution of $Ba(OH)_2$,containing a trace
of alkali and colored by phenolphthalein, ~~by a stream of CO₂~~ . Even
though the last trace of color were removed by acid, they re-appeared,
as stated above, almost at once. The presence of the alkali in the so-
lution undoubtedly sets up a reaction with the $BaCO_3$,assisted
mechanically by the stirring, whereby $Ba(OH)_2$ or Na_2CO_3 ,or both,are
reformed, and the color re-appears. We discovered this effect of
the contaminating alkali in the baryta, in the course of our experi-
ments, but later found that the same thing had been pointed out by
Gill[11] and by Seyler[31] . The presence of an excess of $BaCl_2$,which
could be made by titrating the solutions in the chamber first,would
prevent this effect on the alkali, but is not practicable in our

work. It may be asked here why the first neutral point could not be taken, but a mechanical peculiarity in the method of titrating, which will be explained later on under the head of titration, makes *(Note 12).* it impossible to take the first neutral point obtained and lastly demands that the final end reaction must remain permanent. Alkali is nearly always an impurity in Barium Hydrate even though marked "C.P." That of Kahlbaum's manufacture proved to be the only alkali-free sample from several we obtained in the market.

Note 5 -- On account of the alkaline silicates present even in the very best of Bohemian glass, some recommend that bottles for alkaline solutions should stand for 24 hours filled with Seiler's Solution[32] (H_2SO_4, $K_2Cr_2O_7$, and water), or with a mixture of 1% H_2SO_4 and $HCrO_4$[33]. However, we have found, that, even with every such precaution, it is impossible to preserve weak standard baryta solutions at one strength for more than two weeks at a time, because of reactions with the glass. Consequently the baryta solution has to be restandardized, now and then, taking the HCl as the true standard. Letts and Blake[34] have practically obviated this difficulty by coating the inside of the bottles with paraffine wax. Glazed porcelain vessels suggest themselves as the best. But as restandardization of the baryta solution at any time is a very simple matter, we retained the bottles.

Note 6 -- To filter a standardized baryta solution, we have found it best to fit a clean, dry bottle with a funnel containing a double filter. The baryta solution is siphoned over from the first bottle and allowed to fall into the filter, using either a short piece of rubber tubing furnished with a clip, or a stop-cock, to control the flow. During the filtering, a paper is fitted over the top of the funnel to exclude air currents as much as possible. The air to replace

the baryta in the first bottle is passed through a CO_2 trap before gaining access to the bottle. Bo so doing,a perfectly clear solution of approximately standardized baryta can be obtained. The baryta has to be accurately standardized later,when the supply bottle containing it has been put in place at the apparatus.

Note 7 -- We,at one time,tried a layer of paraffine oil on the surface of the baryta solution in hopes of better preserving its constancy of strength,but with no success. This result was substantial proof that the subsequent weakening in the baryta solution was due to materials which it dissolved out of the glass. It is recognized, of course,that paraffine oil may not be strictly impervious to CO_2 , but in this experiment,in which the regular CO_2 trap was attached to the bottle as well,the addition of the oil did not retard the usual weakening of the baryta solution in the least. This is another proof of the efficiency of the traps. Furthemore, we observed that if any CO_2 came in contact with the surface of the baryta, it formed floating "islands" of Ba CO_3 precipitate on the surface. Hence this is a key to the presence of CO_2 in the air above the baryta solution.

Note 8 -- The presence of CO_2 in the acid, or of $BaCO_3$ in the baryta,can be quite easily demonstrated. After the bottles have been connected with the distributing system and the relative strengths of the solutions accurately found,any appreciable $BaCO_3$ in the baryta, whether in the form of a precipitate or in solution (as it is slightly soluble),can be shown by carrying out a titration in which the acid is added first. As the baryta,containing $BaCO_3$,is added,part of the acid will be used upon the latter,consequently a less bulk of baryta solution will be used to neutralize the acid than normally. The presence of a little $BaCO_3$,however,is no detriment as long as the regular experiments are to be made,for in these the baryta is always added first.----CO_2 in the acid can be told in two ways: (1)the

usual chemical one, in which the end reaction between methyl orange and phenolphthalein is compared. (This is not strictly accurate, as claimed by chemists, since methyl orange has much stronger acid properties than phenolphthalein, and, consequently is more sensitive to alkalies[27,35]). (2) By connecting the air exit tube of one absorption chamber to the spiral of another, dropping 20-30 c.c. of the suspected acid in the first chamber, and baryta in the second, then passing the air current through for an hour or more. Any CO_2 dissolved in the acid will be slowly removed by the stream of air bubbles passing through it and caught by the baryta beyond, which, when titrated will show a corresponding loss. See Note 10. below.

Note 9 -- Cohn[25] gives four or five methods of detecting impurities in phenolphthalein as well as complete directions for making up the solution, its application, etc. -- We found the sample made by Eimer and Amend, New York, the most satisfactory of several specimens examined for purity. This sample was even more delicate than Trommdorff's Table (Cohn, page 222) gives, viz. - 1/2 c.c. of a 1% alcoholic solution required 1/2 c.c. of an N/100 KOH solution to produce distinct redness. We found, that, with 40 c.c. of solution and 4 drops of a 1% alcoholic solution of the indicator, 1 drop (=.07 c.c.) of an N/100 $Ba(OH)_2$ or HCl solution produced an easily detectible change in the end reaction.

Note 10 -- Phenolphthalein possesses the properties of a very weak acid and is very sensitive to CO_2 which will show "acid" with this indicator. Hence it is essential that all acids used with the indicator should be free of dissolved CO_2 . In ordinary work this is usually avoided by boiling the solutions while titrating, thus driving off the CO_2 from the acid. Since this is impracticable in our work, the only alternative is to make up the acid absolutely free of CO_2 and

protect it thereafter with CO_2 traps. It remains then of one strength and may be used for titration in the cold with great accuracy (in the closed system). None of the previous observers in this line of work seem to have considered this point, as all expose their HCl to the atmosphere. To illustrate the fallacy of this[36]: The solubility of CO_2 in weak mineral acid depends upon three things -- the strength of the acid, the temperature, and the amount of CO_2 in the air, or in the vapor phase contiguous to the acid. Therefore, presuming that the strength of the acid remains constant, the amount of the CO_2 dissolved in it still depends upon the temperature and the amount of CO_2 in the atmosphere. If it were possible to maintain these constant, there is evidence to show[37] that it requires from one to three days for the CO_2 equilibrium in such a system to become constant, and before the operator could feel certain that his HCl was constant in strength to phenolphthalein from one day to the next. Bearing these facts in mind, suppose that the distilled water used to make the HCl solution had stood for some time exposed to the air and had reached its saturation point for CO_2 (at ordinary temperatures water will dissolve nearly its own bulk of CO_2[38]). The addition of HCl will drive off a certain amount of the CO_2 . Any subsequent rise in temperature will do likewise. Suppose the bottle containing the "standard" acid is now supplied with a siphon and a CO_2 trap. No more CO_2 can gain access to it. But as the bottle is emptied, a CO_2 vacuum is formed in the air in the bottle above the solution which remains. A CO_2 partial pressure of zero supposedly exists in this vapor phase which is therefore negative to that in the solution below and gradually the CO_2 is given off by the solution to re-establish an equilibrium of the gas between the solution and the air. Consequently the "standard" acid becomes weaker to phenolphthalein. -- On the other hand, if the acid has been made up free from CO_2 and is then left

exposed to the atmosphere,it will at once take up CO_2 and become more "acid" to phenolphthalein. From this will be seen the great importance of guarding against CO_2 in weak solutions of HCl when they are to be used for titrating in the cold with phenolphthalein as an indicator.

Since methyl orange is a stronger acid than CO_2 ,it may be used to determine neutrality between a mineral acid and a fixed alkali regardless of the presence of CO_2 . The first named indicator,however,could by no means be substituted for phenolphthalein in our work,because it is only about one-fifth as sensitive[27] and because it will not indicate neutrality in the presence of suspended $BaCO_3$ precipitate, -- a solution of baryta containing the precipitate will remain alkaline to methyl orange until enough acid has been added to dissolve up all the precipitate.

A long series of experiments has convinced us that solutions as weak as N/100 have no hydrolytic action upon the phenolphthalein[27] ,though we recommend not to add the indicator to a solution until just ready to titrate.

Note 11 -- If precaution is not taken to cut off the last drop of the solution sharply,this will be a source of considerable error in the estimation,for the drop left hanging has been measured but not used. One whole drop is the least that can be used,and amounts to about .07 c.c. With an N/55.5 solution,the end reaction can be estimated to a half drop. Although there might be some advantage in smaller drops,perfect droppers for the same are harder to obtain, and any exposure of the baryta dropper to CO_2 would soon plug it up with $BaCO_3$ precipitate. If such should occur with our apparatus,it could be removed by means of a copper wire bent like a J and inserted through the top tube of the chamber (photograph 2, I).

Note 12 -- The effect of the precipitate on the walls of the spiral has been carefully studied. After about one c.c. of CO_2 has been absorbed,the precipitate becomes easily visible. It will be found heaviest in the lower bulb of the spiral,gradually disappearing higher up. It gives a dull look to the mercury in that part of the spiral. This precipitate has the power to take up a certain amount of any solution,neutral or alkaline,which may come in contact with it. When the spiral has become heavily coated,as much as one-half c.c. of baryta solution may be taken up and held back by it in spite of the two or three flushings given the spiral during a titration. This,of course,would cause a serious error in an experiment. Whether the precipitate holds back the solution by the power of **adsorption**,or whether the honey-comb fashion,in which it is undoubtedly built up,simply retards diffusion through it,is not known. When such a precipitate has been allowed to collect,the only method of getting a baryta solution all out of it,is to let down the neutral solution,after titration,and then keep the air bubbling through it. After five to ten minutes,a coloring of the solution shows some of the baryta has been regained, probably by diffusion. This should be brought up to the chamber,neutralized,and again let down. This process may have to be repeated for an hour before all of a measured quantity of baryta,previously admitted, can be regained,and before the continual slow re-appearance of color will cease. As this is impracticable in the course of an experiment,the precipitate must be removed frequently--generally after 2 - 3 c.c. of CO_2 has been caught,which is equal to using up 10-15 c.c.baryta in toto. During the last periods of a long experiment,a little more time must be given for flushing.---

Although Blackman recommends the use of fairly strong acid to remove the precipitate from the walls of his chambers,we have found this a rather laborous task and use instead, a few c.c. (10 - 15) of the

standard acid. Only a few moments is required. A slow current is used to remove the CO_2 . When the precipitate has disappeared, the greater part of the now weakened acid is removed by the usual outlet (diagram 4, P) the remainder neutralized with a few c.c. of baryta,and the spiral carefully flushed with the neutral solution. This may appear to be a needless waste of the standard acid,but as more baryta is always used up in the course of an experiment than acid,it serves to even up the solutions.

Note 13 -- The shape of a chamber from which it is intended to remove a gas by displacement is of great importance where speed is concerned. The simple tubular style with inlet and outlet at either end is the best form. A spherical form,used on one occasion, having inlet and outlet tubes at opposite sides,pocketed the gas in the lower portion of the chamber for an incredibly long time.

Note 14 -- We have used the ordinary Geissler glass stop-cocks, 2 - 3 m.m. bore, some of those connected with the air passages being mercury seal. However we do not consider this latter improvement of much advantage,as,oftentimes,mercury will slowly work into the vaselined surfaces and make the cocks very difficult to turn. Furthermore,if the cocks are not set horizontally with the handles standing perpendicularly (and this cannot always be done),mercury is continually leaking from them. If the plain style of cocks are made with the plugs correctly ground and given a liberal supply of vaseline (where used for air currents) they may be placed in almost any position and never leak. A defective cock is easily detected by noting the appearance of air lines in the vaselines surfaces. Unless it is intended to attach a vacuum pump to the muscle chamber (when the cocks on either side would have to be mercury seal)no such cocks are needed throughout the whole apparatus.

Bibliography.

1.   Liebig -- <u>Arch. f. Anat. Phys. u. wiss. Med.</u>, 1850.

2.   Fletcher -- <u>Jour. of Physiol.</u>, London, 1898, vol. 23 pp.10-99
     Same journal, 1902, vol. 28, pp. 474 - 498.

3.   Hermann -- "Stoffwechsel der Muskeln", Berlin, 1867.

4.   Metteuci -- <u>Ann. de chim. et phys.</u>, Paris, 1856, Ser. 3,
     tome 47.

5.   Valentin -- <u>Arch. f. physiol. Heilk.</u>, Stuttgart, 1857.

6.   Tissot -- <u>Arch. de physiol. norm. et path.</u>, Paris, 1894, tome
     6, p. 936, and 1895, tome 7, p. 847.

7.   Schafer -- "Text Book of Physiology", vol. I, p. 911.

8.   Blackman -- <u>Phil. Trans.</u>, London, 1895, vol. 186, p. 485.

9.   Stanley -- Thesis, "On the Separation of Motor and Trophic
     Nerves", Univ. of Ill. Library, 1902.

10.   Pettenkofer -- Sutton's "Volumetric Analysis", 1904, 9th ed.,
      pp. 98, 103.

11.   Gill -- <u>Analysist</u>, 1892, vol. 17, p. 184.

12.   Blackman -- <u>Proc. Roy. Soc.</u>, London, 1895, 2nd vol., p.,162.

13.   Fletcher -- <u>Jour. of Physiol.</u>, London, 1898, vol. 23, p.18.

14.   Stanley -- Thesis, "<u>On</u> the Separation of Motor and Trophic
      Nerves", Univ. of Ill. Library, 1902, p. 19.

15.   Stanley -- Thesis, "On the Separation of Motor and Trophic
      Nerves", Univ. of Ill. Library, 1902, p. 40.

16.   Fletcher -- <u>Jour. of Physiol.</u>, London, 1898, vol. 23, p. 13.

17.   "   "   "   "   "   "   "   "   "   19.

18.   "   "   "   "   "   1902   "   28, " 497.

19.   "   "   "   "   "   1898   "   23, "   85.

20.   Stanley -- (see 15) p. 40.

21.   Eimer and Amend -- "Cat. Chem. and Phys. App.",1899, #8657a
      and #8658.

22. Eimer and Amend -- "Cat. Chem. and Phys. App.", 1899, #8136.

23. Sutton -- "Volumetric Analysis", 1904, 9th ed., p. 43.

24.   "   --   "   "   "   "   "   p. 49.

25. Cohn -- "Indicators and Test Papers", 2nd ed., 1902, p. 126.

26. McCoy -- Amer. Chem. Jour., 1903, vol. 29, p. 437.

27. Sutton -- "Volumetric Analysis", 9th ed., 1904, pp. 38, 41.

28. Old Winkler Method.   For description see McCoy, Amer. Chem. Jour., 1903, vol. 29, p. 444

29. Fletcher -- Jour. of Phys., London, 1898, vol. 23, pp. 27, 98.

30. Fletcher -- Jour. of Physiol., London, 1898, vol. 23, p. 79.

31. Seyler -- Analyst, 1897, p. 312.

32. Seiler -- See Lee's "Microtomist's Vademecum,"   , ed., p.

33. Mann --

34. Letts and Blake -- Proc. Chem. Soc., 1896, p. 192.

35. Cohn -- "Indicators and Test Papers", 2nd ed., 1902, p. 114.

36. Cameron and Briggs -- Jour. of Phys. Chem., 1901, vol. 5, p. 546.

37. McCoy -- Amer. Chem. Jour., 1903, vol. 29, p. 446.

38. Just -- Zeit. phys. chem., 1901, vol. 37, p. 342.

---:: Finis ::---