(3)

RESPIRATION OF CHIRONOMUS LARVAE

I

Relations of Chironomus Larvae to low Oxygen Tensions

by

Robert C. Hendrix
Summer 1941

THE RESPIRATION OF CHIRONOMUS LARVAE

Relations of Chironomus larvae to low Oxygen Tensions

Apparatus

In order to study the respiration of Chironomus larvae, which live under supposedly anaerobic conditions in the profundal region of Douglas Lake, methods for keeping the animals in an oxygenless environment for an extended period of time were necessary. It was necessary to remove the oxygen from the water used and to provide against its replacement during the experiment. Two methods have been devised for procuring these conditions.

Plate I shows the apparatus for the vacuum de-oxygenation method. The air is removed by an aspirator attached to a high pressure water line. A manometer placed in the system shows the pressure obtained. The vacuum chamber consists of a vacuum dessicator so arranged that it can be shut of from the rest of the system. Some of the experiments were carried out with the animals in water in the bottom of the dessicator. Others were done with individuals isolated in small bottles placed on the porcelin plate. The conditions will be described fully with the description of each experiment.

In order to check on the effeciency of the apparatus in removing dissolved oxygen from the water, the apparatus was modified so as to allow chemical analyses to be made in the vacuum. This modification is illustrated in Plate II. The reagents needed are placed in upright tubes which lead into the vacuum chamber. These are closed off with screw calmps while the evacuation is being carried out. When the tests are to be made, the stop co cock leadin to the aspirator is closed, and the reagents are allowed to run into the vacuum chamber. They are actually forced in by atmospheric pressure. If care is taken to prevent all of the reagent from running into the chamber, no more oxygen will be admitted than is dissolved in the reagents. The procedure and results of these tests will be discussed in connection with the accounts of the experiments.

It became obvious early in the series of experiments that the decrease in pressure had physical,if not chemical, effects nn the animals aside from those caused by lack of oxygen. It was therefore necessary to prepare an oxygenless medium for the tests whereby the effects of low oxygen tension could be separated from those of the low pressure. To this end an apparatus was constructed for the preparation of oxygenless water by boiling. The purpose of the apparatus was to allow the water to cool without comming in contact with the air after it had been boiled. Plate III shows the construction of the equipment. As the water boils all valves are closed except the steam outlet valve. As soon as the steam stops comming from this valve, it is closed,and the valves controlling the air intake system are opened. The condensation of water vapor in the flask draws air in through this system. The air is drawn through a column of pyrogallic acid or similar reagent which takes up the oxygen, provided the air is drawn slowly enough through the solution. The water is then siphoned off into bottles with ground glass stoppers. These are filled from the bottom and overflowed so that all the water which has come in contact with the air is washed out.

Chemical Tests

Exp. 1t. The following test was performed as a check on the efficiency of the vacuum de-oxygenator. Reference to Plate II will assist the reader in following the procedure.

The aspirator was allowed to run full force for four hours. It was then stopped and the stop cock between it and the vacuum chamber was closed . Afew c.c. of $MnSO_4$ were run into the chamber which was then shaken to distribute the reagent. The KI-KOH was run into the chamber. These two reagents must not flow through the same tube, because the precipitate will clog the system. Upon the addition of the KI-KOH to the chamber a white flocculent precipitate was formed. This was dissolved by the addition of concentrated H_2SO_4. After the precipitate was dissolved air was let into the chamber, and it was opened. Starch solution was then added to a sample of the water. If there had been oxygen dissolved in the water, the end product of the above series of reactions would have been iodine, which would have turned the starch blue. No color was produced when the starch was added, therefore there was no oxygen dissolved in the water in the vacuum chamber. That is, there was none that could be detected by the method described.

Exp. 2t. The question arose as to whether boiling actually removed the dissolved oxygen from thw water or not. Therefore the following experiment was performed.

100c.c. of water were boiled for five minutes. While it was still boiling 1c.c. of $MnSO_4$ was added followed by 3c.c. KI*KOH. A precipitate was formed which was dissolved with conc. H_2SO_4. The Water was boiling while these were being added. When the precipitate was dissolved starch solution was added as a test for iodine. No iodine was present, therefore there was no oxygen dissolved in the water as far as could be determined by this method. Boiling removes practically all of the oxygen.

Exp. 3t. Water was boiled as above, allowed to cool, then siphoned into bottles with ground glass stoppers, care being taken to wash out all that had been exposed to the air in the filling process. This was tested chemically as above and was found to contain a fairly high concentration of dissolved oxygen. The oxygen dissolved in the water while it was cooling in the open flask.

Exp. 4t. Water was boiled as above and siphoned into the bottles while still giving off steam. The bottles had been warmed to prevent their breaking. Upon cooling the water contracted and drew air in around the stopper. This water, when tested, showed a fairly high concentration of oxygen.

Exp. 5t. The apparatus illustrated on Plate III was built as an attempt to procure oxygenless water. The technique of handling this device is described in the section on apparatus. Water treated by this method was tested and found to contain moderate amounts of oxygen. This method, as it stands, will not produce oxygenless water.

Experiments on Chironomus Larvae

Note: Observations are recorded at the time they were made. The times are recorded as hours and minutes after the start of the experiment. Example: 7:09 indicates 7hours and 9 minutes.

Exp. 1a. This experiment was performed in a chamber of the type shown on Plate II without the attachments for chemical work. 12 animals were placed in about 2cms. of water in the bottom of the chamber. These animals had been washed twice in lake water to remove organic detritus and were placed in lake water for the experiment.

Shortly after the pump was started all animals rose to the surface. At the same time gas began to effervesce from the water.

0:20 All animals loose activity.
4:00 Animals very inactive, showing sluggish movements.
7:00 Experiment discontinued due to leakage of air.

Exp. 2a. 5individuals selected at random were washed twice in lake water and put into a large amount of water in the vacuum chamber, which was the same type used in exp. 1a. At the same time 5 animals were placed in a dish of lake water which was left open to the air to serve as a control.

0:0 Pump started
0:06 Effervescence from water. Pressure 32mm. Hg.
0:10 Animals rose to position just under the surface film.
Large amounts of plankton were discovered in the water used. Not examined.
0:20 Animals start to secrete gas in large quantities. One began to show loss of redness in one entire segment. Pressure 16 mm. Hg.
0:30 Pressure 11 mm. Hg. Temperature 15.5 C. Vapor pressure 13.1 mm. Hg.
0:55 All the color has left one segment of the animal mentioned above to be fading. This animal is very inactive and does not respond to the agitation caused by rising bubbles striking it.
1:07 The faded animal became active and seemed to be more faded following the activity.
1:14 The unfaded animals were very deep red, much darker than controls. 3 animals very active at intervals. Faded one inactive.
1:20 All active except faded one. Both ends of this animal very deep red. Control animals do not rise to the top of the water.
2:00 Pump stopped and valves closed. Pressure 9mm. Hg. Temperature 17 C. Vapor pressure 14.5 mm. Hg.
All active except faded one. All dark red wherever any color remains.
2:16 Slight movements of faded individual.
2:40 One animal turning a rusty, brownish red.
2:47 The brown color became quite appearant and the animal became clear and transparent.
2:54 All very sluggish. Faded one showed only occasional weak movements. The segment that faded first is now pale green.
3:22 Another individual had turned rusty red.
3:46 This last individual showed a complete fade out in one segment and a partial fading in a few posterior segments.
3:55 One of the dark red individuals fell to the bottom of the chamber. It was very active on the bottom, then it floated up to the surface and sank back again. These sinking and rising motions continued for 27 minutes. At times the rising and falling occured several times in one minute.. As the animal rose it was seen to be covered with small bubbles of gas,, which passed off as the animal reached the surface. Then it would sink again. After a short period on the bottom the bubbles appeared again, and the animal rose to the surface. This animal retained the deep, bluish,red, color.
4:27 The dark red animal which had not been rising and falling secreting gas. Became very active.
4:42 This animal secreting gas bubbles under the chiton. Had mottled appearance.
4:49 This individual showed fading in the 4th segment from the anterior end.
4:51 Animal which had been rising and falling was turning rusty brown.
4:57 All except second faded animal active.
5:22 The first individual to turn brown was quiet and rigid.
5:24 The two individuals which were last to fade were inactive. The first one to fade was active.
5:32 The two brown animals active, the three faded ones inactive.
7:18 The three faded and the first brown animals were very sluggish. The The other brown was moderatly active.
12:24 Second brown animal very darkly colored.
22:31 Experiment discontinued. Faulty equipment.

Exp. 3a. Five Chironomus larvae were washed twice with lake water and then put into the vacuun chamber with 200c.c. of well water. The chamber was of the same type as was used in Exp. 1a and 2a. Five animals treated as above were placed in 200 c.c. of well water open to the air as controls.

0:00 Pump started
0:05 Three animals had risen to a position just under the surface film. Pressure 60mm. Hg. Temperature 18 C.
0;07 Effervescence of gasses from the water. Pressure 46.5 mm. Hg. Temperature 18 C.
0:09 Effervescence from the animals. Pressure 32 mm. Hg. Temperature 18 C.
0:11 All test animals much darker red that the controls.
0:16 Two animals turning rust brown, almost orange.
0:27 Two animals fading in the 4th segment from the anterior end.
0:30 One of above fading in three segments posterior to the 4th. Remaining color same as controls.
0:32 One of the brown individuals fading in the 4th segment.
0:39 The animal described at 0:30 turning brown. The color is about the same intensity as that of the controls, but is not as clear.
0:43 Pressure 7.5 mm. Hg. Temperature 19 C.
0:47 One animal dark, bluihs red. 3 faded with remaining color rusty red. One unfaded . Rusty red.
0:54. The unfaded brown individual fading.
1:03 All faded animals very quiet. Showed occasional movements.
1:12 Two faded animals and the dark red one moderatly active.
1:15 Pressure 7 mm. Hg. Temperature 20 C.
1:16 Most faded animals least active. Very little effervescence from water. Some from animals.
1:19 Two faded animals very quiet. One had color completly gone from anterior end, the posterior end being dark red. Sharp division between the two zones. Anterior end is free of fluid, and the posterior is full. The division line is a meniscus. It did not remain at a constant level but fluctuated slightly.
1:35 No effervescence from water, some from animals.
1:37 Faded animals inactive, dark red one active.
1:54 Pressure 6 mm. Hg. Temperature: 21.5 C.
2:02 All animals very active and effervescing. Dark red one especially active.
2:05 Pressure 6 mm. Hg. Temperature 22 C.
2:38 All alive, and all but one faded one moderatly active. Very slight effervescence from animals.
2:57 Animal described at 1:19 as having lost all color in anterior end had a small thread of reddish, viscous, liquid draining from the mouth.
3:00 Above animal gave several violent movements and became quiet. Fluid still draining from the mouth.
3:25 Above animal shows occasional movements. Posterior end dark red. Anterior end faint, pale green.
3:35 Pump stopped.
4:18 Pressure 9.5 mm. Hg. Temperature 20 C.
All animals moved when the wall of the chamber was tapped except one faded one.

4:52 Pressure 13 mm. Hg. Temperature 20 C.
All alive
4:54 Pump started.
5:16 Pressure 6 mm. Hg. Temperature 19.5 C.
6:11 All respond to tapping except extensivly faded animal described at 3:00.
7:29 Extensivly faded animal described at 3:00 had begun to replace the red pigment. The first two segments had almost completly recovered their color. The color replaced is dark red.
9:43 Pressure 13 mm. Hg. Temperature 19.5 C. Vapor Pressure 17 mm. Hg.
All alive.
9:46 Pump started.
10:45 Pressure 4mm. Hg. Temperature 18 C.
All animals alive. Faded ones are rebuilding pigment.
Experiment discontinued. Faulty equipment.

Exp.4a. Five individuals were prepared for the test and five for the controls exactly as described in the preceding experiment.

0:00 Pump started.
0:03 Effervescence from water.
Pressure 61 mm. Hg. Temperature 17.5. C.
0:04 Effervescence from animals.
0:05 Animals at position immediatly under the surface film. Controls did not do this. Test animals very active.
0:41 Effervescence from water slight.
Pressure 8mm. Hg. Temperature 17.5 C.
One animal faded completly in 4th and 5th segments and slightly in 6th and 7th.
Another had lost all color from 4th segment and is dark red at either end.
Another had faded evenly, although not completly, from the 4th segment back to the last few posterior segments. Remaining color differs very little from control.
The two remaining animals showed dark red anterior ends with the rest of the body colored like controls.
1:09 No effervescence from water except near animals. This may be from animals. General color of tests darker that that of controls.
1:32 All animals fading in middle of body. Ends dark red.
Pump stopped.
Pressure 6.5mm. Hg. Temperature: 18 C.
3:05 One animal had lost all color from anterior end.
Pressure O.K.
4:31 All alive. Most extensivly faded with unfaded parts dark red.
Pressure 7mm. Hg Temperature 18 C.
6:08 All alive
Pressure O.K. Pump started
6:18 Pump stopped.
7:05 All alive. None very active. All more or less faded.
10:52 Animal which had lost color at anterior end as described at 3:05 has begun to replace color.
20:51 Three animals had sunk to bottom. One of these was very quiet and did not respond when the glass was tapped. Only one showed much fading.

38:39 All animals quiet. Did not respond to tapping.
Pressure O.K.

The animals were taken from the vacuum chamber and were placed in oxygenated water open to the air. The controls were all alive and a bright clear red. Two of the test animals were faded and had no rigidity, the other three were darkred and very stiff. None of these animals showed any signs of recovery in the oxygenated water, Evidentally they had been killed by the treatment applied to them.

Exp. 5a. Five animals were washed twice with well water, and then one placed in each of five small screw top "Packer" bottles. These bottles were filled with well water and closed without any air. They were then placed in a vat of water which completly covered them. The well water used contained some dissolved oxygen.

0:00 Animals sealed in bottles.
2:49 #1 showed slight fading in the 4th segment.
Others normal.
40:35 #1 Anterior portion a pale rusty red with the 4th segment almost colorless. Posterior portion dark red. Animal stretched out stiffly and showed very slight movements.
#2 An even dark red color. In tightly coiled position untill disturbed whereupon it became quite active.
#3 Like #2.
#4 An even, pale, dull, red,color. In a tightly coiled position untill disturbed, whereupon it became quite active.
#5 An even, pale, dull, red, color. It lay stretched out quietly untill disturbed, whereupon it became quite active.
43:35 #1 Stiff and quiet. A cloudy opaque red except for the last two or three segments which were dark red.
Others as described above.

48:35 #1 No motion even when shaken. Color as at 43:35.
Others as described above.
51:49 #1 Stiff and quiet. Color as above.
#3 Fastened to the bottle with silk. Was coiled and difficult to excite.
All others as above.

75:49 #1 Definitly dead!
#2 and #3 Dark red and hardest to excite.
#4 and #5 Light red or brown and easier to excite.
135:49 #2 and #3 Dark red color. Alive.
#4 Probably dead.
#5 Dead.
140:49 #2 and #3 Alive.
Others dead.
166:54 #2 and #3 . No longer resting coiled up but lying at full legnth. Very little motion when disturbed.
168:49 #2 and #3 Coiled up. Very inactive and slow to respond to disturbance. Evenly colored dark red.
214:04 #2 and #3 Alive. Resting at full legnth, but curl up when moved. Deep dark red.

220:59 #2 and #3 Moderatly active without being disturbed. Dark red.
221:56 #2 Inactive except on vigorous.disturbance.
#3 Active upon slight disturbance.
223:39 #2 Coils and uncoils when disturbed. Otherwise inactive.
#3 Active, no excitor needed.
237:41 #2 and #3 Show little or movement even when disturbed.
239:19 #2 and #2 Alive. No movement unless disturbed and not much then.
Dull red.
249:11 #2 and #3 Alive.
308:38 #2 Dead.
#3 Alive and quiet unless disturbed.

Exp. 6a. Procedure as in Experiment 4a.

0:00 Pump started.
0:04 Effervescence from water. Animals Just under surface film.
0:16 Effervescence from animals.
0:45 All at surface. Very quiet.
1:10 One animal fadind in the midregion.
Others brownish red. All darker than controls.
1:23 Three animals active, two quiet. Faded one quiet. Others brownish red.
1:53 Two inactive, others active. All brownish red.
2:43 Three animals inactive. two active. One active animal fading in 3rd or 4th segment. One quiet animal brown. Others dark red.
Pump stopped.
4:35 All alive, three inactive.
Pressure O.K. Pump started.
5:52 All alive. Pump stopped
7:50 Three active, two inactive. All quiet when undisturbed.
9:32 All alive. All inactive unless disturbed. Two did not respond to tapping.
Pump started.
12:35 All but one respond to bright light. This one is faded in 4th segment.
Pressure O.K.
12:38 Pump started.
12:45 Pump stopped.
22:30 All evenly colored brownish red. One inactive, others more or less active.
Pump started.
22:37 Pump stopped.
27:32 All but one respond to tapping. One on bottom.
27:33 Pump started.
27:56 Pump stopped.
27:57 Two animals on bottom, do not respond to tapping. One at surface did not respond. Two that responded are inactive except when disturbed.
29:17 Two show no motion when chamber is tapped, others move only when disturbed. One quiet animal was at the surface, the other on the bottom. The active animal on bottom was deep red. The inactive one(which moves occasionally) was brown. All at surface were brown.
31:11 Two on surface respond to tapping. One of these had posterior end at surface and hung downward from there. One at surface quiet. Two on bottom do not respond.

35:16 One has dropped to bottom, not one which was hanging from surface. Is active.
40:23 Pump started.
40:27 Four on bottom inactive except when disturbed. One did not respond to tapping. One on surface did not respond.
40:30 Pump stopped.
49:55 Four on botton, three active, one motionless. One motionless on surface. Motionless animals are brownish red. Active animals vary from that to dark red.
50:07 Two bottom animals quiet even when disturbed.
53:22 Bottom animals and one on surface showed no response to tapping.
55:15 One bottom and one surface animal showed no response to tapping.
All dark, brownish, red.
Pump started.
55:22 One bottom animal very active, another moderatly so.
55:24 Another botton animal showed great activity for a short time.
55:25 Pump stopped.
56:52 three of four bottom animals quite active. The other would respond to neither tapping or bright light.
59:35 Three of bottom animals would not respond to tapping. Other active without tapping. Surface animal quiet, probably dead.
62:27 only one responded to tapping. All are brownish red.
64:03 Two bottom animals responded to tapping. One active without stimulus. Surface animal is dead.
64:07 Pump started.
64:15 Pump stopped.
75:13 All animals appeared to be dead.
82:40 All dead, one lost color, others brownish red.
87:25 All placed in oxygenated water open to air. One was entirly disentegrated, others redish brown. Four recovered and regained bright red color.

Exp. 7a. This experiment was performed in a vacuum chamber of the type illustrated on Plate II. One Chironomus larva was placed in each of eight small, numbered bottles. They were arranged as follows:
#1 half full of well water, open.
#2 full of well water, closed with no air bubble.
#3 half full of well water, open, contained small amount of detritus from lake bottom.
#4 Full of well water, closed with no air bubble, contained small amount of detritus from lake bottom.
These four were placed in the vacuum chamber.
#5 Half full of well water, open to air.
#6 Half full of water, open to air, contained small amount of detritus from lake bottom.
#7 Full of well water, closed without air, not put in vacuum chamber,
#8 Full of well water, closed without air, not put in vacuum chamber, contained small amount of detritus from lake bottom.

0:00 Pump started.
0:05 #1 At surface, effervescing.
#3 and detritus at surface, effervescing.

0:13 #1 Very active and effervescing.
#2 At surface, hidden behind cap of bottle. Effervescing.
#3 At surface, detritus on bottom. Vigorous effervescence from animal, water, and detritus.
0:20 Pressure 20mm. Hg.
#1 Brownish red.
#2 Out of view.
#3 Brownish red.
#4 In close contact with detritus, dark red.
#5 On bottom of container, bright red.
#6 In close contact with detritus, bright red.
#7 On bottom, active, bright red with silvery sheen.
#8 Like #7.
0:32 #1 Faded in 4th segment.
#4 At surface and hidden by cap of bottle.
0:40 #1 Extensivly faded.
#5 At surface of water.
1:20 #4 Had fallen to view and had been closely associated with detritus. Same color as controls. Rose to surface at this time.
1:25 #3 Reddish brown with silvery sheen.
#4 At bottom.
#5 and #7 Same color.
1:30 #2 Fell to bottom and rose slowly without movement. Repeated several times.
1:35 #1 Very still.
All controls approximatly the same color.
1:45 #2 On bottom and active.
#1 Changed position.
#3 Blown up on side of bottle by explosive bubble from detritus.
#4 On bottom.
#2 Rising from bottom.
3:35 #1 Active; faded.
#2 Out of view.
#3 Dried up.
#4 Out of view.
#5 At surface
#6 At surface and same color as #5, i.e., bright red.
#7 Like #6.
#8 Like #6.
Experiment discontinued on account of leak.

Exp. 8e. Set up as in Exp. 7a with the containers arranged as follows:
In vacuum chamber:
#1 and #2 Half full of water, one larva.
#3 and #4 Half full of water, one larva, detritus from lake bottom.
Open to air:
#5 Half full of water, one larva.
#6 Half full of water, one larva, detritus from lake bottom.

0:00 Pump started.
0:02 #1,2,3,4, at surface.
Pressure 70mm. Hg.

0:03 Effervescence from animals and water.
Pressure 24mm. Hg. Temperature 24°C.
0:12 Animals very active. Vigorous effervescence from animals and water.
0:13 Pressure 0mm. Hg.
1:08 #1 and #2 Fading mostly in 4th segment, but some in posterior segments.
1:18 #4 slightly and evenly faded over several middle segments. This appeared to be a change to an opaque red rather than a loss os color.
1:28 Pump stopped.
2:08 #2 Slightly faded in 4th segment.
Others as above.
2:25 #6 Just under surface film.
#:33 #2 Faded again.
All animals in chamber much darker than controls. Control #6 somewhat duller that #5.
5:19 #4 Mottled with faded spots at irregular points.
Others as above.
6:19 #3 Thrown up on wall of bottle by explosive bubble from detritus. Dark red.
#4 Large bubbles exploding from detritus.
#1 and #4 Very still.
#2 Active.
Pump started.
6:29 Pump stopped.
6:46 #1 No response when chamber is tapped.
#2 Responds to tapping.
#4 Blown up on side of bottle.
7:04 #5 At surface.
Experiment discontinued, Faulty equipment.

Exp. 9a. Procedure as in 7a and 8a with the containers arranged as follows:

One larva in each bottle.
In vacuum:
#1 and #2 Half full of water.
#4 and #5 Half full of water, detritus from lake bottom.
Open to air:
#6 Half full of water.
#7 Half full of water, detritus from lake bottom.

0:00 Pump started.
0:03 Effervescence from water. Pressure 65mm. Hg. Temperature 22°C.
0:05 Alltest animals and detritus at surface. Animals effervescing and active.
0:16 Detritus on bottom. Bubbles had formed on the detritus, causing it to float. When these burst, it sank.
0:38 #2 and #5 Faded in 4th segment.
Effervescence from animals, but not from water.
#1 and #2 Darker than controls.
#4 and #5 Same color as controls.
1:08 #1 Darker than control.
#2 and #5 Faded, brownish.
#4 Brown.
Pressure 15mm. Hg. Temperature 23°C.

1:37 #1 darker than control.
#2 and #5 Faded.
#4 Brownish.
2:00 Pressure 16mm. Hg. Temperature 23°C. Pump stopped.
4:15 #1 Dark red, not faded. Hanging with anterior at surface.
#2 and #5 Faded alike.
#4 Brown.
All inactive. Moved when exposed to bright light.
Pressure 19mm.Hg Temperature 27°C. Pump started.
4:20 Pump stopped.
4:57 All as above.
6:25 #1 No response to bright light.
Others as at 4:15.
7:05 All Alive
#4 Fading.
#1 Showed no fading.
8:50 All respond to bright light.
11:50 #1 On bottom.
#4 and #5 Hanging from surface.
23:30 All except #2 on bottom.
#1 Dull brown.
#2 At top, faded, inactive, no response to light.
#4 and #5 Brownish red.
#6 Dark red, very inactive.
#7 Bright red, active.
24:10 #2 and #5 Quiet.
26:45 #2, #5, and #6 No response to bright light.
28:50 #1 Slightly active, much darker than #7.
#2 and #5 Quiet, no response to bright light.
#4 Brown, very sluggish.
#6 Quiet, no response to bright light.
#7 Active, bright red.
Pressure O.K.
47:10 Pressure 17mm. Hg. Temperature 20°C.
#1 On bottom, dullred with whitish cloudy blotches.
#2 Completly decolorized, water reddish.
#4, #5, and #6 Brownish, opaque, dead.
#7 Bright clear red, alive.
50:10 #1 Alive, motionless on bottom. Responds to bright light.
61:15 All test animals dead.
Pressure and temperature O.K.
#6 Dead.
#7 Alive, bright red.
Bodies of test animals placed in open dish of oxygenated water. There were no recoveries within 10 hours.

Exp. 10a. Procedure as in experiments 7a, 8a, and 9a. The containers were arranged asfollows. One larva in each bottle
In vacuum:
#1 and #2 Half full of Water.
#4 and # 5 Half full of water, detritus from lake bottom.
Open to air:
#6 Half full of water.
#7 Half fullof water, detritus from lake bottom.

0:00 Pump sterted.
0:03 #1 At surface.
Pressure 70mm. Hg.
0:04 All test animals at surface.
Pressure 70mm. Hg.
Effervescence from water and animals.
#4 Carried detritus to top.
4:45 #1 Dark red.
#2 and #4 Faded in 4th segment.
#5 Brown.
5:10 #4 Slight fading posterior to 4th segment.
6:20 #1 Dark red.
#2 and #4 Extensivly faded.

9:33 #1, #2, and #5 Onn bottom.
#4 Rising and falling.
#5 Showed tendancy to float to top, but never got there.
#1 Showed same tendancy.
12:37 #5 Mots of time on bottom.
12:43 #5 At surface.
16:55 #2 No response to bright light.
All uniform color, a dullred of about the same intensity as controls.
Controls are bright and transparent red, Test animals are dull and opaque.
Pressure holding at 16mm. Hg. Would not go below.
26:10 All animals appearantly dead.
#1 and #2 Deep red, rust color.
#4 Opaque rust.
#5 Hidden by moisture condensed on walls of chamber.
Pressure 16mm. Hg.
29:40 #1 Responded to bright light.
#2, #4, and #5 No response to light.
37:45 No response to light. All dead.

Discussion

Position of the Animals in the Water

In all of the experiments performed with the vacuum chambers, it was noted that soon after the vacuum pump was started the test animals rose to a position just under the surface film of the water. This change of position was not due to any definite swimming motion of the animal. Animals kept both as controls and as stock did not come to the surface, but it was noted that, when,in collecting the larvae, the bottom material was dumped from the dredge in to the tub, many individuals did rise to the surface of the water. This could not all have been due to mechanical agitation.

Most of the experiments in which the pressure was noted at the time the animals came to the surface show that they did so at pressures between 60 - 70 mm. Hg. Table I gives a summary of these data.

When the pressure is decreased the solubility of a gas is also decreased. In this case gases probably come out of solution in the body fluids of the animals and form gas pockets inside the body. These increase the bouyancy, and the animal rises to a position under the surface film. Evidently the bouyancy is not increased enough to force the animal through the film. Great amounts of gas are given off at these low pressures, and this hypothosis requires only that some of this gas be retained as bubbles by the animals.

After a period of time at low pressures the animals sink again to the bottom of the containers. They would sink, then rise and sink again. The force of the vacuun was evidently great enough to remove, in time, the accumulated bubbles of gas retained in the animal. This decreased the density of the mass and permitted it to fall through the water. If the gas was still being secteted, the animal would float upwards again, either because

some of the gas was retained or because bubbles stuck to the outside.

In Experiments 7a and 8a the controls rose to a position just under the surface film. In these experiments the controls were placed in small Packer bottles as were the test animals. Since the controls of other experiments, which were placed in larger amounts of water, did not rise to the surface, it is probable that the small amount of water had something to do with this action. It may be that this is an effect caused by the excretory products of the animals.

This phenomenon, or as much of it as is made clear by these experiments, seems to be the direct result of the lowering of the pressure and not to be directly connected with the lack of oxygen.

Loss of Color

The summary of the data on fading and the phenomena connected with it given in Table II shows briefly the order of occurance of the fading and the times and conditions connected with the recovery of color. It is obvious that no general statement concerning this small and incomplete collection of data can be made. It appears that in the majority of cases the time at which the individuals began to show fading or loss of color is approximatly the same. Many exceptions occur to this however.

It is possible that the fading is caused either by lack of oxygen or by decreased pressure. The total pressure difference between the test animals and the controls was never more than one atmosphere and was seldom that much. When the animals were collected they underwent a pressure change of about two atmospheres. They were brought up from about 20 meters of water, the pressure of which is about two atmospheres. No fading was noticed at that time. One of the animals in Experiment 5a, in which there was no decrease in pressure although there was a decrease in the oxygen tension, showed

rather extensive fading. Four others placed under the same conditions showed no fading. Many animals placed in the vacuum chamber showed no fading. Faded animals were noted to be very inactive. It was also noticed that strenuous movements increased the fading. This may indicate that the fading is largely due to a reduction of the haemoglobin. If little oxygen were available the animal would not be very active, and, conversly, activity would increase the reduction of the haemoglobin. It is probable then, judging by the observations made, that the lack of oxygen causes the fading. The possibility that the excretory products of the animals caused the effect is ruled out because controls under the same conditions except pressure and oxygen tension did not fade.

The fading noticed seems to have been of two different types. One is an actual change in the coloration of the body fluid, while the other involves a loss of the fluid. The latter type arreared in Experiments 3a, 4a, and 9a. The former type is probably due to a chemical change in the blood, as has been pointed out. It was not possible to study this change chemically. The loss of fliud may have been a physical effect of the low pressure, but this explanation leaves the question as to why this should occur over a change of one atmosphere when it did not occur over a change of two atmospheres. A possible answer to this is, that, since the loss of fluid occured in only three animals out of the many studied, the total change of three atmospheres caused tissue damage in already weakened individuals. An objection to this is that the animals in Experiments 3a and 4a eventually replaced all of their color. The animal in Experiment 9a, however, eventually became completly decolorized. It had died before all the color had gone.

Of the 25 animals which showed fading, 14 regained some type of red color while being maintained at the same, or in some cases more rigorous

conditions than those at which they had faded. Since nothing is known concerning the chemistry and physics of these changes, no conclusions are warrented. However there is the possibility that the animals do become aclimated to the low oxygen tension, or other factors causing the fading, and are able to repair the damage done to the haemoglobin. If this could be proved, it would serve as an indication of the manner in which they adapt themselves to the to the rigorous conditions in the stagnation zone.

Color Changes

The change of color from a bright, clear red to a dull, rather bluish red is probably due to the reduction of the oxyhaemoglobin. The bright red oxyhaemoglobin occurs when there is a adequate supply of oxygen, and the dark red reduced form occure in situations in which there was no available oxygen.

All but very few of the animals eventually became a reddish, rust brown when under treatment in the vacuum chamber. It was also noted that the individuals as drawn up from the stagnation zone had a very similar color. Most of the animals recovering from fading built up this color. This may indicate the change in the respiratory pigment necessary for the transport of oxygen when it is present in small quantities. The brown animals were rather inactive, so it may be assumed that the lack of oxygen was causing a slowing of the metabolic processes.

Fatalities

Table III summarizes the times of death of the experimental animals and gives a description of the animals at or near the time of death.

Experiments 4a and 6a were performed in the same vacuum chamber, therefore the reste of exhaustion are fairly comperable. The times of death do not coincide. Experiments 9a and 10a were run in a different chamber in which

the rate of exhaustion was higher than in the other type. The times of death coincide fairly well.

It will be noted that most of the animals were brownish red at death. Reference to the experimental data will show that most of these had been brown for many hours before death. Therefore the brown color is not a mark of death, nor is it a change which will bring about death immediatly. One animal died and became completly colorless. This was appearantly loss of fluid and was probably due to the physical effect of the low pressuse. In many cases the individuals had been faded, but most of them recovered their color before de death. Only two animals died while still faded. Three individuals died while they were the deep red color of reduced haemoglobin. They had failed to make the adjustment to brown and lived a much shorter time (relative to the rate of exhaustion) than any of the others.

The animals of experiment 5a lived longer that the others because they had to use up the supply of oxygen before they felt the lack which the others experienced almost immediatly. The variations in the times of death in experiment 5a are probably due to individual or specific differences.

The only justification for supposing that these deaths were due to lack of oxygen rather than low pressure is that the animals of Experiment 5a, which were not subjected to low pressure, showed the same changes in thee respiratory pigment and in the rate of activity as did the ones subjected to the low pressures.

Effects of Detritus on Changes in the Animals

In Experiments 7a, 8a, 9a, and 10a small amounts of detritus from the lake bottom were placed in the containers with the animals. This detritus was screened out of the bottom mud at the same time that the animals were and was mostly fiberous in nature. When the pressure was lowered great

[illegible] were given off, [illegible] heavily, from the detritus. [illegible] have [illegible] bearing on the problem, but to

amounts of gas were given off, sometimes explosivly, from the detritus. The nature of this gas may have an important bearing on the problem, but it could not be determined.

Animals in contact with this detritus showed all the movements, fadings, recovery of color, and changes of color that have already been described. The animals died more quickly in the detritus than did those which were not exposed to it. The controls, open to the air, showed exactly the reverse, that is, the animals without detritus died sooner than those with it. Appearantly the detritus decomposing under anaerobic conditions gave off a substance that was toxic to the animals. This substance was not produced, or had no effect, when an adequate supply of oxygen was available. This result does not seen consistant with the animals live directly in the bottom mud in the lake. It may be that, in the lake, the dilution is great enough to nullify the toxicity of the substance. It is also possible that the same conditions obtain at the bottom of the lakeas did in the controls; i.e., there is enough oxygen available to prevent either the formation or the effect of the substance.

General Conclusions

1. It is indicated that the larvae of Chironomus require oxygen to live.

2. There is some adjustment in the chemistry of the blood made by the larvae under low oxygen tension.

3. At very low oxygen tension the bottom mud produces a substance toxic to the larvae.

TABLE I

Position of Animals in the Water

Rise to Surface*		Fall to Bottom*		
Exp.	Pressure	Exp.	Pressure	Time
1a	more than 32mm. Hg.	2a	9mm. Hg.	3:55
3a	60mm. Hg.	4a		20:51
4a	60mm. Hg. (approx.)	6a		27:57
7a	20mm.Hg.	9a		23:30
8a	70mm. Hg.	10a		9:33
9a	65mm. Hg.			
10a	70 mm. Hg.			

* Individuals in the two columns do not correspond.

TABLE II

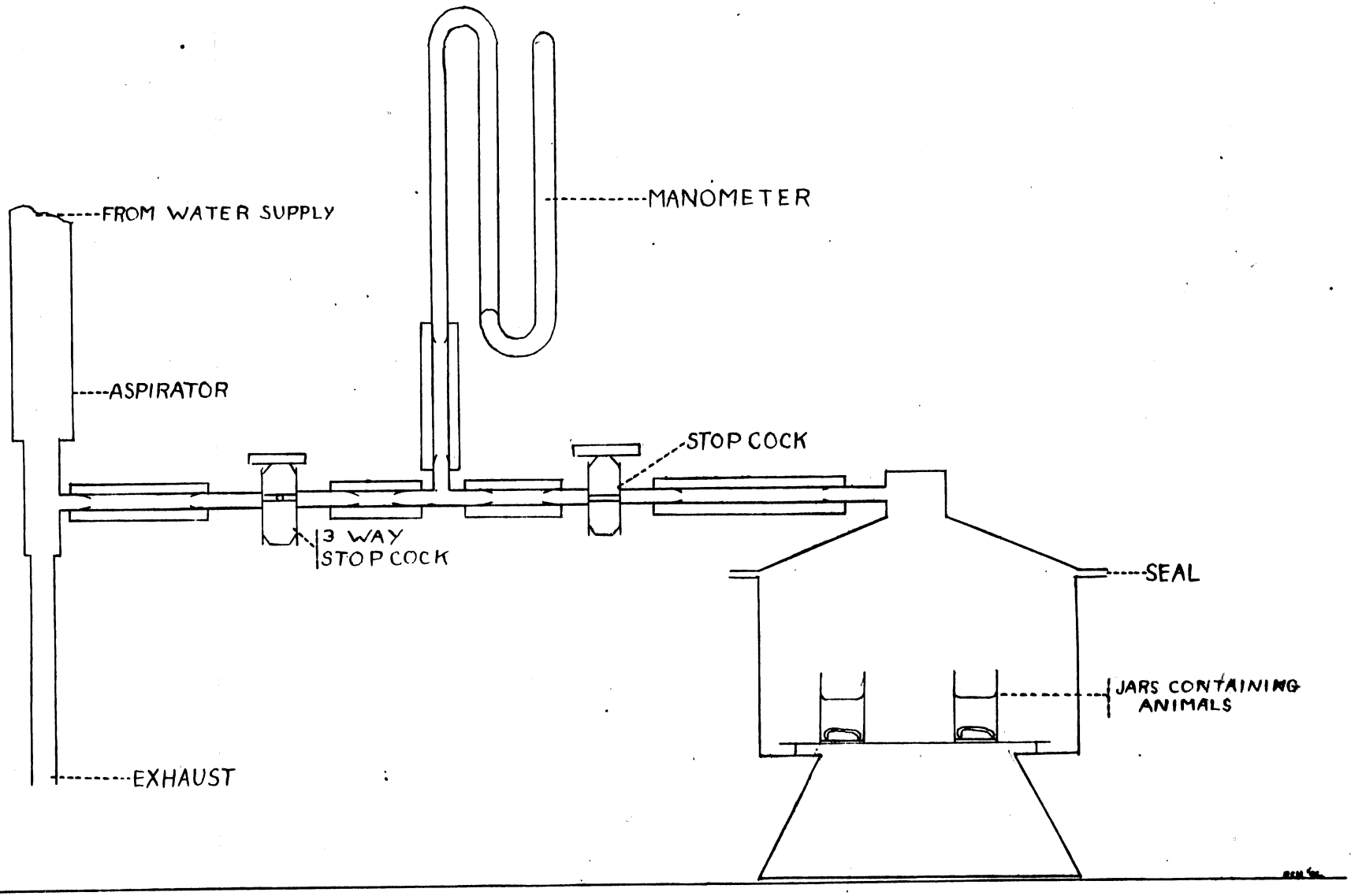
Fading and Recovery

Fading*				Recovery*			
Exp.	Time	Pressure	No.	Exp.	Time	Pressure	No.
2a	0:20	16mm. Hg.	1	3a	7:49	6mm. Hg	1
2a	3:46	14.5mm. Hg	1	3a	10:45	4mm. Hg	3
2a	4:49	-----	1	4a	10:52	-------	1
3a	0:27	----	2	4a	20:51	-------	3
3a	0:32	----	1	5a	43:45		1
3a	0:54	-----	1	6a	29:17	-------	1
4a	0:41	8mm. Hg.	3	9a	23:30	--------	2
4a	1:32	6.5mm. Hg	2	9a	16:55	16mm. Hg.	2
5a	2:49		1				
6a	1:10	-------	1				
6a	82:40(dead)	-----	1				
7a	0:32	-----	1				
8a	1:08	------	1				
9a	9:38	-----	2				
9a	7:05	19mm. Hg	1				
10a	4:45	-------	2				

*Individuals in the two columns do not correspond

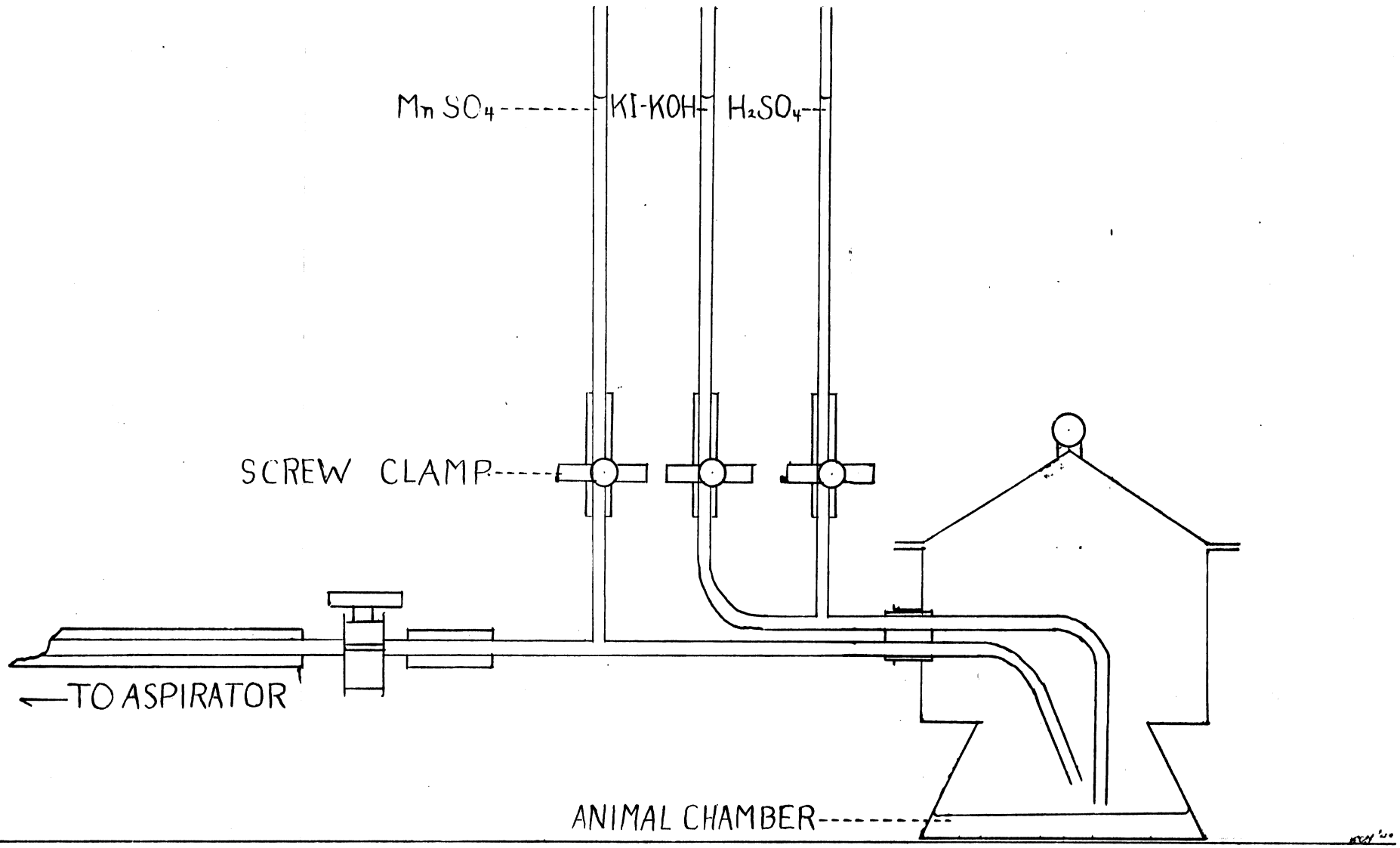
TABLE III
Fatalities

Exp.	Time	No.	Description
4a	38:39	5	All had been fading before death. 2 still faded, flabby. 3 deep red, stiff.
5a	43:35	1	Had been faded before death. Now opaque rust, deep red at posterior end.
5a	135:49	2	Brownish rust color.
5a	308:38	1	Brownish rust color.
6a	82:40	1	Complete loss of color.
9a	37:45	2	Dull brown.
9a	61:15	1	Dull red with whitish blotches.
10a	29:40	3	Opaque rust.
10a	37:45	1	Brownish red.



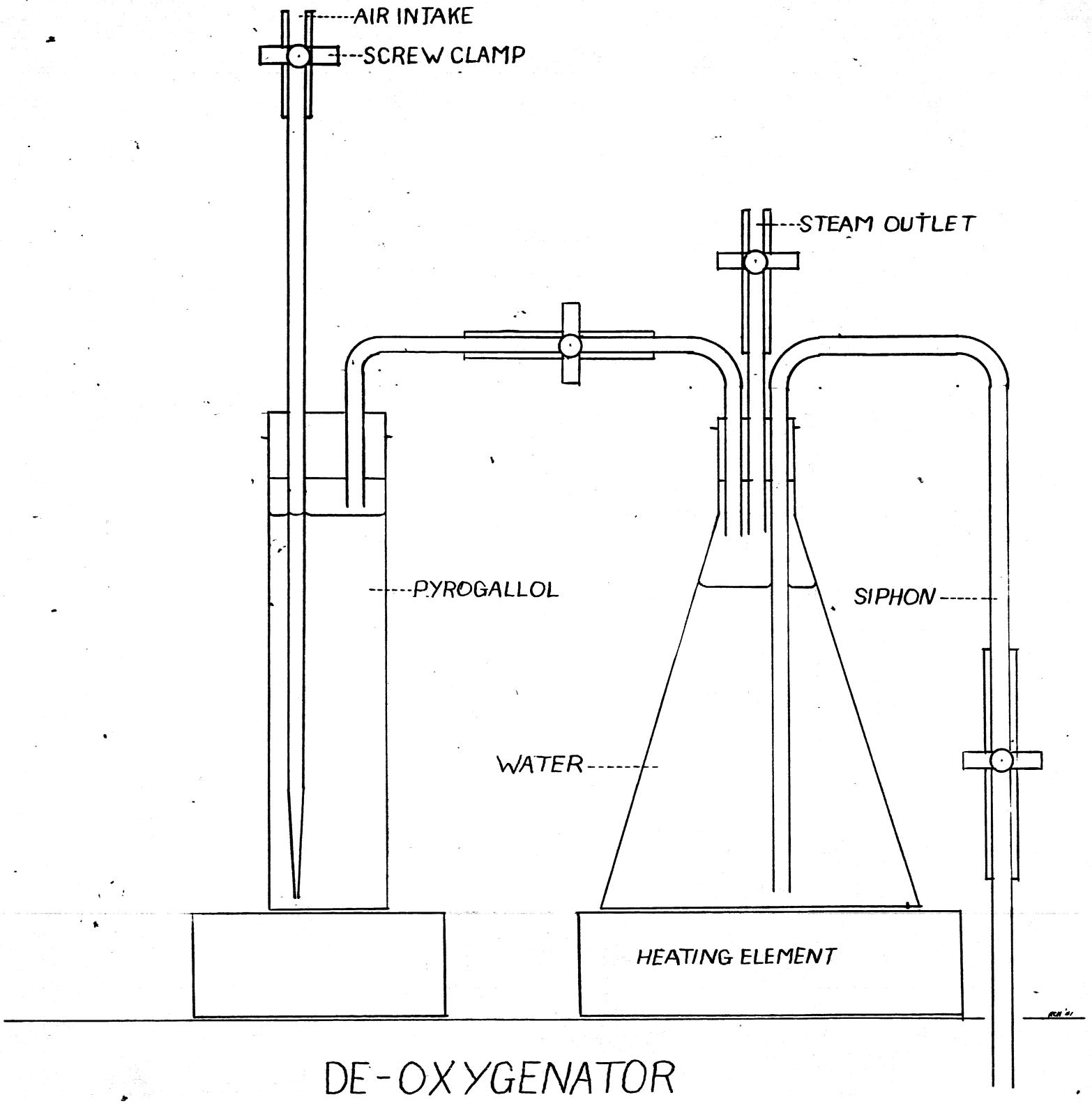
VACUUM DEOXYGENATOR

PLATE I



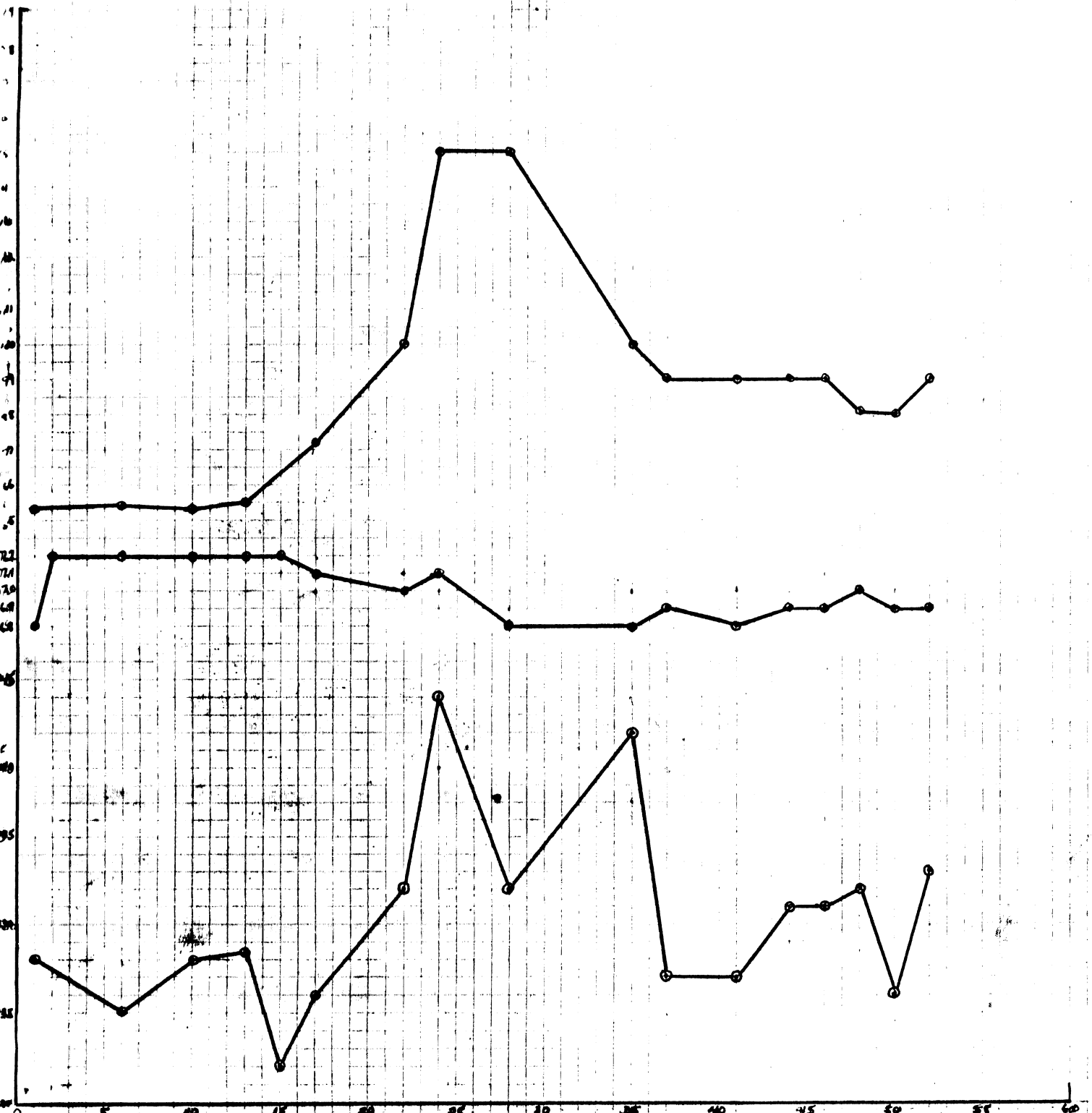
VACUUM DE-OXYGENATOR
MODIFIED FOR CHEMICAL ANALYSIS

PLATE II



DE-OXYGENATOR
BOILING METHOD

PLATE III


0
5
10
15
20
25
30
35
40
45
50
55
60