THE RELATIVE REACTION WITHIN LIVING MAMMALIAN TISSUES.

IX. ON THE TISSUE REACTION AS INFLUENCED BY INHALATIONS OF CO₂ AND BY OVERBREATHING.

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In preceding papers from this laboratory the influence upon the tissue reaction of injections into the circulation of weak solutions of acid and alkali has been recorded, and the relation existing between the blood changes and those in the tissues during the course of an acidosis induced in this way has been described (1). We have now sought to determine the effect upon the tissues of breathing high concentrations of carbon dioxide, and of the overventilation that results from forced respiration.

The Inhalation Apparatus.

White rabbits were used. The general technic of vital staining with phenol red and of appraising the reaction of the blood and the changes in the color of the body surface have already been detailed (2). The buffer solutions used in the wedges and to determine the reaction of the blood differed slightly in pH, as determined with the potentiometer, from the calculated values. In charting the results corrections have been made for the differences, whereas in the protocols the figures as originally read off are recorded.

A special apparatus was necessary, for the purpose of the inhalations of carbon dioxide. It is portrayed in Text-fig. 1.

The gas from the storage cylinder escaped into one or the other of two 20 litre bottles graduated in half litres and about half full of water weakly acidulated with hydrochloric acid. The pressure under which the gas passed into the one bottle was sufficient to force water over into the other through the large tube connecting the two, and the only way of escape for the carbon dioxide already in this other was through a tube which led to the inhalation mask fixed upon the animal's muzzle. By means of a special snap clamp (X) acting, now on the tubes A, and again on B, the direction of flow from the storage cylinder could be alternated to
the bottles at will without interruption of it. An observer with a stop-watch controlled the speed at which gas passed to the inhalation mask. One litre per minute was the ordinary rate.

The mask was made out of a short broad bottle from which the bottom had been cut. Across the opening thus provided a diaphragm of rubber was stretched with a hole in the center for the rabbit's muzzle. There was a continuous current of gas through the bottle, and the space in it was small, to prevent rebreathing. The tube through which the gas escaped was long in order that there might be no sucking back of air into the mask on inspiration. To ensure a snug fit of the rubber diaphragm all hair was removed from the animal's muzzle with a sodium sulfide solution. The mask was held in place by a strip of adhesive tape passing around the back of the head, and there was never any leakage between it and the skin. Care was taken not to interfere with the circulation to the ears.

Five experiments were performed with a mixture containing 21.08 per cent of CO₂, and approximately 22.09 per cent O, and 56.83 per cent N. A single set of observations were made with 37 per cent CO₂, 22 per cent O and 41 per cent N. For the analyses of the gas mixtures, as supplied in pressure cylinders, we are indebted to the kindness of Dr. C. A. L. Binger.

In four of the five experiments just referred to, urethane was employed as anesthetic. The blood specimens for the pH determinations were procured from a
femoral vein. In the case of the remaining animal, which received no general anesthetic the small incision necessary to bare the vein was made after infiltration of the tissues with novocain. To aspirate blood Luer needles bent at a reentrant angle were used, as in the previous work. It was essential that the circulation of the leg furnishing the blood should not be interfered with, so no thong or other tie was placed about it. The vein could be punctured again and again in the groin without any important bleeding or thrombosis, if the needle was thrust slantingly through its sheath and pressure applied for a few moments after the aspiration.

In several of the experiments a minimum dose of urethane was employed and a far better respiratory response to the acidosis was obtained than in the animals more heavily anesthetized by its means and given hydrochloric acid (3).

**The Acidosis on Breathing 21 Per Cent of CO₂.**

The protocol of Experiment 1 need not be given since the facts are better illustrated by the later work.

**Experiment 2.**—Male rabbit No. 2, of 1650 gm., given 3.3 cc. of 50 per cent urethane subcutaneously into the neck at 9.20 a.m., and 1 cc. more at 10.35, into the peritoneal cavity. 11.00—10 cc. of 4 per cent phenol red isotonic with 0.9 per cent NaCl and at pH 7.4 was injected into an ear vein. 11.50—Rabbit for some time on the warm pad. *Surface hue* slightly less purple than *eugenia red* (Ridgway); by wedge method between pH 7.5 and 7.6. Skin folds compressed between slides are pink by transmitted light with faintest yellow admixture; ear cartilage pale pink. 11.54—*First blood specimens taken.* 12.00—*Surface hue* between pH 7.5 and 7.6.

12.01—*Mask adjusted and gas run in.* The respirations at once mounted from 40 to 88 per minute, much exaggerated; but almost as rapidly they lessened in frequency again.

12.05—*The surface hue* generally has become yellower, between *jasper red* and *light jasper red.* No indication of spotting. 12.07—Respirations 56 per minute, regular. 12.10—*Surface hue* yellower than *jasper red.* Skin folds orangy-yellow. 12.12—*Surface hue coral red.* 12.41—*Surface hue light coral red.* Skin folds pronouncedly orange-yellow; ear cartilage light yellow. 12.48—*Surface hue* by wedge method, pH 7.2. 12.51—*Second blood specimens taken.* 12.55—*Surface hue* by wedge, pH 7.2. 12.56—*Surface hue* still light coral red. During this long inhalation period the respirations have been much exaggerated but never stormy. Their rate has lessened from 56 to 48 per minute.

12.57—*Mask removed.* The respirations at once became much less ample, 52 per minute. Practically at once, too, the *surface hue* became pinker, the change being very definite by 12.59.
1.01—Animal stirred, as it had not during the inhalations. 1.03—Skin fold much less yellow. 1.08—Surface hue now approaches old rose. 1.12—Surface hue by wedge, pH 7.4. 1.14—Ear cartilage more pink than yellow now, while skin fold is as pink as before the inhalations. 1.19—Surface hue between jasper pink and old rose, pH 7.4 by wedge. 1.26—Third blood specimens taken. 1.30—Surface hue by wedge pH 7.4—; between light jasper red and jasper pink. Observations discontinued. The respirations varied between 52 and 40 after the mask was off; they were quiet.

Experiment 3.—Performed on afternoon of same day as Experiment 2 and upon the same animal, which was still unconscious owing to the urethane. 2.52—Given 8 cc. of phenol red into the same ear vein as before. 3.17—Surface hue slightly purpler and deeper than jasper red, by wedge method pH 7.5+. 3.19—First blood specimens taken. Ear cartilage is light pink; skin folds, compressed, are pink, with faintest yellow tinge. 3.23—Surface hue by wedge, pH 7.5+.

3.28—Mask adjusted and inhalations begun. The respirations, which had averaged 48 per minute, quickened greatly at once but soon slowed again, to 62, much exaggerated.

3.32—Surface hue yellower. 3.33—Skin folds much yellower. Some surface patching of color. 3.35—The general color is jasper red, with some coral red patching over chest and abdomen. The patches are irregular, only a few centimeters across. They have the general character of those seen in outlying acidosis. 3.43—The coral red patches have become confluent, everywhere driving out the rosy hue. A skin fold is bright orange-yellow but the color of the ear cartilage has not changed. 3.53—Surface hue pH 7.2+ by wedge method. There is a definite yellowing of the ear cartilage. The circulation in the ear is excellent. 4.00—Surface hue still that of pH 7.2+. 4.02—Second blood specimens taken. 4.07—Surface hue between coral red and light coral red; by wedge method pH 7.2+. Ear cartilage yellow. During the inhalations the respirations gradually slowed from 62 to 44 per minute, markedly exaggerated always.

4.08—Mask removed. Immediately the breathing became stormy and quick for a few moments, but then quieted and slowed to a rate of 48 per minute and gradually, by 4.30, to 32 per minute. The color of the animal at once began to change back to the normal, being definitely more ruddy by 4.11. The change was generalized.

4.13—Surface hue between jasper red and light jasper red; skin fold pink, with but a slight admixture of yellow; ear cartilage still yellowish. 4.19—Surface hue between light jasper red and old rose. 4.25—Third blood specimens taken. 4.28—Surface hue by wedge, pH 7.4. 4.30—Animal stirring slightly.

Observations discontinued. 50 cc. of warm water given by stomach tube. Next morning the rabbit was in excellent condition, eating.

The buffer solutions used in the wedges and to determine the reaction of the blood differed slightly, on test with the potentiometer, from the calculated pH values. Corrections have been made for the differences in charting the results, whereas in the protocols the figures originally read off are given.
As the protocols and the chart show (Chart 1) there was in these experiments not only a great alteration in the reaction of the blood but one in the extravascular reaction as well, when the animal inhaled a gas mixture containing 21 per cent CO₂ and the normal quantity of oxygen. The changes in the surface color indicative of an increasing acidity began practically at once, and, progressing rapidly, reached a maximum some little time before the inhalations were discontinued. When this had been done an immediate return toward the normal took place, at first rapid, then more gradual. In Experiment 3 there was, as the general acidosis developed, a patching which resembled that of outlying acidosis.

Experiment 4.—Male rabbit No. 4 of 1700 gm. given 4.5 cc. of 50 per cent urethane into the peritoneal cavity at 9.20 a.m. and 10 cc. of 4 per cent phenol red into an ear vein at 10.04. 10.25—Staining is even and deep; animal on warm pad, deeply anesthetized. 10.48—Surface hue not quite so purple as eugenia red; by wedge method at pH 7.5. 10.54—First blood specimens taken. 10.57—Surface hue still at pH 7.5. A skin fold is pink with slightest tinge of yellow; ear cartilage pink.

11.08—Inhalations begun. Immediately the respirations increased in rate and amplitude.

11.12—Surface hue is yellower. 11.14—Surface hue now jasper red save for a longitudinal streak about 4 cm. wide extending from ensiform nearly to symphysis, which is still purply red. 11.15—Skin fold more yellow. Respirations still markedly exaggerated but of nearly the same rate as before the inhalations were begun, 48 as compared with a previous 44 to 46 per minute. The rate altered practically not at all from now until the mask was removed. 11.23—Dubious yellowing of ear cartilage. Good circulation in the ear. 11.29—Surface hue between jasper red and coral red. 11.33—Skin folds are much more yellow; and so too is the ear cartilage. 11.40—Surface hue generally is between coral red and light coral red. The median streak of differing color has disappeared. 11.48—Circulation to ear is excellent yet cartilage is orange-yellow, as also are the skin folds. 11.52—Surface hue by wedge method pH 7.2+. Second blood specimens taken. 11.59—Surface hue at pH 7.2; yellower than coral red. Color of skin folds and cartilage orange-yellow.

12.00—Mask removed. Respirations temporarily increased in amplitude and frequency, being 60 to the minute at 12.01 but soon becoming quiet and slowing to 40. Practically no change took place in the surface hue for a long time, however. At 12.37 it was still between coral red and light coral red; and at 12.40, by wedge method, at pH 7.2.

12.46—Third blood specimens taken. 12.49—Surface hue by wedge pH 7.2. 2.00 p.m.—Rabbit still deeply unconscious, an even light jasper red. Skin surface
Extravascular changes in reaction resulting from inhalation of CO₂

Chart 2.

- pH
- % Plasmol red
- % CO₂
- % O₂
- % N₂

Hrs.

0 1 2 3 4
is warm.  2.07—*Surface hue* between pH 7.3 and pH 7.4.  Skin folds still much yellower than normal.  Ear cartilage has decolorized too far for appraisal of the hue.  2.18—Fourth blood specimens taken.  2.24—*Surface hue* by wedge between pH 7.3 and pH 7.4.  2.25—*Surface hue* between *light jasper red* and *old rose*.  Skin fold still is yellower than “normal.”  Animal has not stirred at all.  5.00—Color still a medium light pink.  Animal deeply unconscious.  Observations discontinued.  50 cc. of warm water was given by gavage and next day the rabbit was in excellent condition.

The injection of urethane into the peritoneal cavity resulted in a deeper anesthesia than when the same dose was given subcutaneously (Experiments 2 and 3).  The rabbit never moved during the observations, and the staining persisted for an unusually long period.  But more important was the persistence of acidosis after the mask had been taken off (Chart 2).  Comment has already been made (4) on the action of urethane to lessen or prevent the compensatory respiratory response that is usually called forth by an acidosis.  As result of this occurrence the acid products of metabolism tend to heap up within the organism to a greater extent than would follow merely from the introduction of acid from without.  In the present case the extravascular acidosis indicated by the surface hue was not unusually marked; and hence one can scarcely attribute its persistence to an unusual accumulation of acid metabolites.  Doubtless the depression of the respiratory activities persisted after the mask had been taken off.

As a check upon the findings observations were now made in the absence of a general anesthetic.

*Experiment 5.*—Male rabbit No. 5 of 1500 gm.  10.25–30 a.m.—10 cc. of phenol red given into an ear vein.  The animal at once began to color up rapidly and evenly.  It was placed on the warm pad and a femoral vein bared with the aid of novocaine.  11.02—*Surface hue* slightly less purple than *eugenia red*; by wedge method at pH 7.6.  11.05—First blood specimens taken.  The animal is quiet.  11.10—Skin flaps are pink, with the slightest yellow admixture.  The cartilage of the ear is deep pink.  11.14—*Surface hue* by wedge still at pH 7.6.

11.15—*Inhalations begun.*  The breathing at once became rapid and stormy but the animal did not struggle then or later.  The respiratory rate rose abruptly from 80 to 110 but gradually fell again, reaching 78 at the end of the inhalation period.  Throughout it the breathing was greatly exaggerated.

11.17—*Surface hue* rapidly losing its purple quality.  Some linear razor marks heretofore not visible are now rendered suddenly prominent by an orange border
to either side of them. 11.20—Surface hue nearing jasper red. 11.23—There is a pronounced spotting over the abdomen, of many orange-red patches about 1 cm. in diameter, scattered on a more ruddy background. The veins are nowhere turgid. 11.25—The patching has disappeared and the acidic hue is confluent save for a broad streak from ensiform to pubis which is still purply red. The streak corresponds with the region where a poorly vascularized aponeurosis underlies the skin. The skin flaps are a marked orange-yellow and so too is the ear cartilage. The circulation in the ear continues excellent. 11.29—Surface hue between coral red and light coral red. The inside of the ear, where a bloodless cartilage is but thinly overlain with skin, is no longer pink by reflected light but yellow. The scleral conjunctiva has turned ruddy orange. There is an excellent conjunctival reflex and the animal appears conscious. 11.38—Surface hue slightly lighter than coral red; the median streak of more purply red has almost completely merged in the general hue. Skin flaps are clear orange-yellow; ear cartilage orange-yellow. 11.44—Surface hue at pH 7.3 by wedge method. 11.49—Second blood specimens taken. 11.50—Surface hue at pH 7.3.

11.50—Mask removed. Immediately the respirations became even more ample than before and the animal appeared excited. The superficial veins over the abdomen and chest became greatly engorged even in the finer ramifications. 11.52—Surface hue redder and conjunctiva has become pink again; but skin flaps are still orange-yellow. 11.54—Animal has now quieted and so too have its respirations. 11.57—Surface hue between eugenia red and old rose. The venous congestion continues. 11.59—The skin flaps have returned to the usual pink with faintest admixture of yellow; the ear cartilage is much pinker. 12.06—Surface hue as at 11.57; by wedge method at pH 7.5. 12.08—Third blood specimens taken. 12.15—The animal has struggled at intervals but remains quiet during repair of the groin incision. The veins over chest and abdomen are still notably congested. 12.17—Circulation in the ears is greatly cut down, and the cartilage is still somewhat yellow both by transmitted and reflected light. 2.40—Animal in excellent condition, very light pink. 3.05—Animal decolorized except for the ears which show a light pink cartilage. The superficial veins are still engorged. Observations discontinued. The animal was let up, when it behaved normally.

The results of this experiment (Chart 3) corresponded in general with those of the preceding ones. The tissue acidosis did not become so great, however, and recovery from it was more prompt, though not quite complete when the observations were terminated.

The Acidosis on Breathing 37 Per Cent of CO₂.

During the latter part of the inhalation periods of the foregoing experiments the surface hue changed but little. It was evident that the
limits had been reached of the extravascular acidosis that could be induced with 21 per cent CO₂. In order to obtain more marked changes resort was had to a mixture containing 37 per cent of this gas.

Experiment 6.—Male rabbit No. 6 of 1500 gm. No general anesthetic. At 10.16 a.m. 10 cc. of phenol red was given into an ear vein and a little later the animal was placed on the warm pad and a femoral vein bared with the aid of novocaine locally. 10.52—Surface hue slightly less purple than Eugenia red; by wedge method at pH 7.6. 11.08—Beginning to decolorize, so 4 cc. more of phenol red given into same vein. 11.23—Surface hue slightly yellower than at 10.52; by wedge method between pH 7.5 and pH 7.6. 11.30—First blood specimens taken. 11.33—Surface hue slightly above pH 7.5. Skin flaps are pink with a trace of yellow, and ear cartilage also. The circulation in the ear is good. 11.35—Mask on and inhalations begun. The animal held its breath for about half a minute, then struggled, soon became quiet, and began to breathe regularly and very deeply. During the inhalation period the respiratory rate gradually lessened from 68 to 42 per minute.

11.38—Surface hue rapidly becoming yellower, nearing Jasper red except along midline of abdomen, where as yet there is little change. 11.40—Ear cartilage much yellower. 11.41—Skin flaps already ruddy orange. 11.42—Surface hue, generally, Jasper red. 11.51—Ear cartilage orange-yellow. 11.52—Surface hue Coral red. 11.53—Animal appears to be completely unconscious; slight rhythmic movements of legs; good conjunctival reflex. 12.00—Surface hue about midway between Coral red and Carnelian red. 12.01—Skin flaps more orange. 12.05—Bloodless ear cartilage yellow by reflected light. 12.09—Surface hue Carnelian red. 12.14—Surface hue by wedge method at pH 7.1. 12.22—Second blood specimens taken. 12.23—Surface hue slightly yellower than Carnelian red; by wedge method pH 7.1 —.

After 12.00 n. the accessory muscles of the neck were called into play during the respirations. By 12.18 these were labored and less extensive. For fear that a gradual failure of ventilation might ensue and complicate the findings the mask was removed at 12.24. At that time there were 42 breaths to the minute. The color of the animal was still changing toward orange. Immediately that the mask was taken off the respirations became much shallower and the rate rose to 82 per minute. The rabbit, previously unconscious to all appearance, raised its head at 12.26 and struggled at 12.27. The ruddy color was now rapidly coming back. 12.28—Surface hue approaching Coral red. 132 respirations per minute, shallow. 12.33—Rabbit quiet; 100 respirations per minute. Surface hue here Jasper red, there Coral red. 12.36—Conscious but quiet. 12.43—Surface hue only slightly yellower than old rose. 12.47—Ear cartilage still definitely yellower than ordinary. 12.48—Skin flaps have the normal pink hue. 12.58—Surface hue is old rose; by wedge method at pH 7.5. 1.03—Third blood specimens taken. 1.05—Surface hue by wedge, pH 7.5. Experiment discontinued. During its course the rabbit had
lost somewhat less than 5 cc. of blood by a slow escape from the femoral vein. When seen next morning it was in excellent condition.

The effect of breathing an atmosphere containing 37 per cent CO₂ with about the ordinary quantity of oxygen was to render the animal unconscious, and to induce a progressive acidosis (Chart 3). Toward the end of the inhalation period there were signs of respiratory failure; and complications from this source would doubtless have occurred had the mask not been taken off. The extravascular acidosis induced was more considerable than with 21 per cent CO₂ and apparently its limits were not reached. The acidosis of the blood became as pronounced as is ordinarily compatible with life, according to other investigators. Recovery was prompt but not quite complete during the brief period of observation.

**Effects of Overventilation.**

As a corollary the influence upon the tissue reaction of overbreathing was studied. It was already known that flaps of living and well vascularized connective tissue become more alkaline when exposed to air (5). So too does the peritoneal lining. With overbreathing sufficient to reduce considerably the carbon dioxide tension of the blood one would expect some change in the extravascular reaction. Such a change was obtained, a definite but not a marked one as would follow from the fact that the induced blood alkalosis was but slight. Six experiments were performed, upon rabbits.

Under general anesthesia, brought about with urethane in all save one instance, the animals were tracheotomized and a limb of a T-tube passed down nearly to the bronchial bifurcation and tied in place. In the exceptional case ether was used during the tracheotomy and the lips of the incision were swabbed with novocaine prior to discontinuance of the general anesthesia, at the time when the experiment proper was begun. The tube that formed the staff of the T was connected with the house suction in some instances and in others left open, while the free limb was connected with a motor-run mechanism devised by Dr. F. L. Gates, whereby air, separately warmed and moistened, was blown continuously or rhythmically into the lungs. The chest of some of the urethanized animals was opened by a bloodless incision down the middle of the sternum and a screw retractor inserted to expose the lungs. The blood specimens were taken from a femoral vein, and the surface hue appraised by the wedge method as usual.
Blowing air continuously for half an hour through a catheter with its opening down almost as far as the bifurcation of the bronchi was without effect on the surface hue of the animal stained with phenol red; and when the stream of air was cut off no apnea ensued. Filling and emptying the lungs by the alternate blowing in of air, and suction upon the tracheotomy tube with the house vacuum resulted in well defined changes but great care had to be taken to control the pressure relations, else pulmonary hemorrhage ensued. The best results were obtained in animals with relatively flexible thoracic walls which permitted of a large expansion and deflation of the lungs. It was found that when the chest was opened the animal developed the signs of a marked outlying acidosis despite the existence of an overventilation as proven by the apnea that ensued when the artificial respiration was stopped. In our opinion this acidosis resulted from a peripheral vascular constriction, secondary to and compensatory for, an interference with the circulation which was in turn traceable to an embarrassment of the heart. This last organ,—no longer provided with its usual orientation and supports,—collapsed upon itself at each deflation of the lungs.

The alterations in the reaction of the blood and tissues were never great; and the surface hue was more difficult to appraise in terms of pH than was the case in the acidosis experiments, because the colors were well toward the alkaline side of the range of phenol red. The colors could not always be recorded precisely in terms of Ridgway's nomenclature because the values associated with alkalosis differed somewhat from his scales. Two protocols will be given.

Experiment 7.—(a) Male rabbit No. 7 of 2075 gm. given 4 cc. of 50 per cent urethane into the peritoneal cavity at 12.02 and 10 cc. of phenol red into an ear vein at 1.08. A few whiffs of ether were administered during the tracheotomy, which had been accomplished by 1.27 p.m. 1.37—Surface hue between eugenia red and jasper red but deeper than either; by wedge pH 7.5-. Skin flaps pink, with faintest yellow admixture.

1.40.—Artificial respiration by pressure and suction begun. The suction was sufficient to cause marked depression at the costochondral junctions and the normal respiratory rhythm was easily overcome. 1.46—On intermitting briefly the artificial respiration an apnea ensued lasting 45 seconds. 2.00—Surface hue deeper than eugenia red; by wedge pH 7.6+. Skin flaps deep purply red, no yellow.

2.23—Discontinued artificial respirations. Surface hue at this time just above pH 7.6 by wedge. Apnea for 70 seconds; then resumption of breathing at 44 respirations per minute. During the suction and compression the rate had varied between 28 and 32. 2.27—Surface hue returning toward eugenia red. 2.39—Surface hue between eugenia red and jasper red. 2.55—Surface hue less purple and slightly lighter than eugenia red. Skin flaps once again show a tinge of yellow in the prevailing pink.

(b) 3.00 p.m. of same day—Surface hue by wedge pH 7.5. 3.01—First blood specimens taken. 3.05—Surface hue by wedge pH 7.5-. Skin flaps colored as before. Respirations 42 per minute.
Artificial respirations begun at 32 per minute. 3.08—Animal decolorizing. Surface hue only slightly darker than old rose. 3.17—Surface hue now much darker and purpler than eugenia red. 3.31—Decolorization proceeds. Surface hue lighter than eugenia red; by wedge midway between pH 7.6 and 7.7. 3.32—Second blood specimens taken. 3.34—Surface hue by wedge between pH 7.6 and 7.7.

3.35—Artificial respiration stopped. Apnea of 50 seconds. 3.37—Respirations 146 per minute, surface hue much less purple. 3.59—Respirations have gradually become slower, now 54 per minute. Surface hue old rose, by wedge pH 7.5. 4.13—Third blood specimens taken. 4.20—Surface hue alizarin pink, by wedge pH 7.4+. At 4.26 the animal was killed with ether and autopsied. It showed two localized pulmonary hemorrhages, 0.5 and 1.5 cm. in diameter respectively, one in each lower lobe.

Experiment 8.—Male rabbit No. 10 of 1600 gm. Given 1.3 cc. of urethane into tissues of back of neck at 12.30; 1 cc. more into peritoneal cavity at 2.00; and a few whiffs of ether during the tracheotomy, which was accomplished by 2.30. Between 2.21 and 2.25 10 cc. of phenol red was injected into an ear vein. The vital staining was even and deep, but owing to time lost in some accessory observations it was necessary to give 3 cc. more of the phthalein, at 3.30, to compensate for the decolorization that was taking place. 3.43—Surface hue slightly lighter than eugenia red, at pH 7.6 by wedge. 26 respirations per minute. 3.47—First blood specimens taken. 3.48—Surface hue, pH 7.6. 3.50—Artificial respirations begun at rate of 28 per minute by alternating pressure and suction. 3.58—The blood in the superficial veins appears much purpler, so that these are more clearly seen. (The like observation was made in other experiments not reported here in detail.) 4.11—Surface hue by wedge between pH 7.6 and pH 7.7. 4.16—Second blood specimens taken. 4.19—Surface hue purpler and lighter than eugenia red, by wedge pH 7.7+. Artificial respirations stopped. Apnea for only 35 seconds. Thereafter the respirations were at first rapid, 140 per minute, and then gradually slowed, to 82 per minute. 4.24—Surface hue lighter and only faintly purpler than eugenia red. 4.34—Surface hue slightly lighter and faintly purpler than old rose; by wedge method pH 7.6-. 4.38—Third blood specimens taken. 4.40—Surface hue by wedge pH 7.6-. Respirations 96 per minute. Observations discontinued. The rabbit was chloroformed for examination, on the same day. No lesions were found other than a liver cocccidirosis.

Changes in the color of the connective tissue and cartilage could not at any time be made out.

While the alterations in reaction produced in these experiments (Chart 4) were not great they were sufficient for the purpose in hand, namely to determine whether an extravascular alkalosis is linked with
the blood alkalosis induced by overbreathing. Such is the case, the alterations in the blood reaction being closely paralleled by similar extravascular ones, as attested by the surface hue. In one of the two experiments the purple hue of skin flaps compressed between slides and viewed by transmitted light indicated the development of a more alkaline reaction of the connective tissue. No such change was to be seen in the ear cartilage.

**DISCUSSION.**

The experiments show that under the circumstances of acidosis due to the inhalation of carbon dioxide, and of alkalosis consequent on a blowing off of the gas, changes occur in the extravascular reaction which closely parallel those in the blood. Without doubt the extravascular changes largely involve the interstitial fluids, but during the acidosis concomitant alterations take place as well in the connective tissue and cartilage, the matrix tissues so readily rendered acid by the intravenous injection of acid solutions. During the alkalosis we noted connective tissue changes only, but this is not surprising since it was slight in degree and of brief duration. In our experience alterations in the reaction of cartilage follow rather tardily upon those of the connective tissue (vide Experiment 3); and this is especially true of the cartilage in the ear of the rabbit, which is almost avascular in the regions best suited to inspection. No observations were made upon the deep-lying organs; but one would expect from the results in rats given hydrochloric acid (6) that under the circumstances of acidosis due to carbonic acid the tendons would also become acid. Whether the reaction would also change in the liver, pancreas, and lymph nodes, parenchymal organs unaffected apparently by even the most extreme hydrochloric acid acidosis, is an interesting question. Quite possibly this would happen. For carbon dioxide manifests abilities to penetrate living tissue far beyond those of other acids (7).

An extravascular acidosis began to develop practically at once on the inhalation of the carbon dioxide, whereas it did not appear until a large quantity of hydrochloric acid had been run into the blood. This was to have been expected since the introduction of hydrochloric acid is compensated for in large measure by the elimination of carbon dioxide through the lungs, a possibility excluded when the gas is
being taken into the body by the pulmonary route. But over and above this difference one may attribute the prompt development of the extravascular CO₂ acidosis to the known ability of the gas to penetrate tissues rapidly (7). Evidence of such penetration was clearly to be seen in the course of our experiments. Where the circulation was unusually good, as for example about the almost imperceptible abrasions due to shaving (Experiment 5), the acidosis developed soonest; and where the vascularization was scanty, as along the line between ensiform and symphysis (Experiments 4 and 5) there it was seen last. The patching with color sometimes witnessed during the course of the inhalations (Experiments 3 and 5), and resembling that of outlying acidosis, was in reality consequent on a diametrically opposite state of affairs, those parts becoming most rapidly acid, and in consequence standing out in orange-red against a red background, which were most accessible to the acidotic blood, not least so.

The continuance of a carbon dioxide acidosis would doubtless lead to some accumulation of acid metabolites here and there in the body; but during the brief period of our experiments little evidence of such an event was obtained. The reaction of the blood and tissues swiftly changed for the better when the inhalations were discontinued, in significant contrast to the persistence of acidosis when hydrochloric acid had been administered. True, the reaction did not quite return to the normal even under the best of conditions (Experiments 5 and 6), and a compensated acidosis may well have been present and have endured for some time. To determine the actual case was no part of our work which had for sole aim the immediate influence upon the tissue reaction of alterations in the CO₂ content of the blood.

The normal reaction of the blood, as obtained from the femoral vein in the present experiments, varied from about pH 7.25 to slightly less than pH 7.4, whereas that from an ear vein, as noted in our preceding work (8), ranged from just below pH 7.4 to nearly pH 7.5. This very considerable discrepancy between the reaction of the two sets of specimens read in precisely the same way and by the same observer was a consistent finding. The fact may be recalled in this connection that the blood had come from very different regions. Since our object was to follow the relative, not the actual, variations
in intravascular and extravascular reaction the problem thus raised will not be discussed.

Hawkins has shown that urethane anesthesia results in an alkalosis (9); yet there can be no doubt that under uncomplicated conditions it tends to prolong the acidosis resulting from the inhalation of carbon dioxide (Experiment 4, Chart 2), as also that induced by hydrochloric acid. There is evidence that this comes about through a change in the respiratory center (10).

SUMMARY.

Breathing an atmosphere that contains the normal amount of oxygen but a large excess of carbon dioxide results in a tissue acidosis as well as one of the blood. The extravascular changes in reaction take place with far greater speed than when acidosis is induced with hydrochloric acid, and they do not persist as in the case of this latter but swiftly disappear when the animal breathes ordinary air once again. The changes parallel closely in magnitude and time those occurring in the blood. The same matrix tissues are rendered acidotic as when hydrochloric acid is administered.

The blood alkalosis that results from a blowing off of carbon dioxide is accompanied by an extravascular alkalosis. Under the circumstances of our experiments the connective tissue became more alkaline than ordinary but no change was noted in the cartilage, a fact to be explained by the slight degree of the alkalosis and its brief duration.

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