# THE RELATIVE REACTION WITHIN LIVING MAMMALIAN TISSUES.

## VIII. On the Course of the Tissue Acidosis Secondary to Blood Acidosis Induced with Hydrochloric Acid.

By PEYTON ROUS, M.D., D. R. DRURY, M.D., and W. W. BEATTIE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 25, 1926.)

In a previous paper (1) the fact has been demonstrated that the reaction of certain tissues can be altered by injecting into the blood amounts of acid or alkali that are compatible with life. Rats vitally stained with a phthalein and receiving dilute hydrochloric acid survive despite marked changes in the color of the connective tissue, tendons, and cartilage. The abnormality in pH thus attested persists for a surprisingly long period. It has seemed possible, by repeating the experiments in larger animals, to learn something of the general relation existing between the reaction of the blood and tissues. Such experiments, upon rabbits, form the main subject of the present paper. But before they are taken up it will be well to give in detail a few observations on the effect upon rats of maximum quantities of hydrochloric acid.

### *Preliminary Experiments on Rats.*

*Experiment I.*—At 9.20 a.m. a male rat of 123 gm., shaved over the body 24 hours previously, was given an intraperitoneal injection of 1.85 cc. of 4 per cent phenol red isotonic with the blood, but at pH 5.6. The animal was then etherized, a cannula connected with a jugular vein, the body immersed in a warm bath of oil, and, at 10.10 a.m., N/6.35 HCl was run into the circulation. The apparatus and general technic were those employed in a previous paper (2). The hue of the rat to begin with was deeper than *jasper red* (Ridgway) and no change was observed until after 3 cc. of the acid had been given, when the shaved surface appeared somewhat yellower. When 5 cc. was in, at 10.37 a.m., it had become *coral red.* The breathing was exaggerated. By 11.05 a.m. 9 cc. had been injected and the animal was slightly lighter than *rufous.* Urine had been repeatedly voided in

large amount, at first red, then orange-red. At 11.10, with 9.5 cc. in, a movement of the animal during the course of the stormy respirations loosened the attachment of the cannula, and the vein had to be tied. The ether was discontinued and the rat taken from the bath. Within 10 minutes it was walking about. The surface tissues, now decolorizing, were still acidotic as shown by a *carrot red* color.

At 1.40 p.m. when the animal had completely decolorized a second injection of 1 cc. of phenol red was given. By 2.30 it was deeply colored, *carnelian red.* At 5.10 the injection was repeated. And now the hue was indicative of a return toward the normal, being between *jasper red* and *coral red.* During the afternoon the rat voided great quantities of intensely orange urine. Next morning, as well as 5 days later, it was in excellent condition. Healing of the neck incision was rapid.

*Experiment II.*—The rat weighed 124 gm. and received 1.85 cc. of the same phthalein solution used previously, soon after which it was etherized and placed in the oil bath. In the course of 1 hour and 40 minutes 14.8 cc. of N/6.35 HCl was run into a jugular vein. Toward the latter part of this period there was marked air hunger. 20 minutes after the injection,—and the ether,—had been terminated, with the animal already up and about, it was given 1.0 cc. more of the phthalein into the peritoneal cavity. Within a minute it died, as if from an embolus,—though none could be found in the pulmonary artery. A clot was present in the jugular below the tie. The color changes during the injection of acid had followed the general course of those noted in Experiment I.

*Experiment III.*—The test was repeated but in this instance the rat of 128 gm. was anesthetized with 1.1 cc. of 20 per cent urethane injected into the subcutaneous tissue of the back of the neck. 15 minutes later it received into the peritoneal cavity 1.9 cc. of 4 per cent phenol red at pH 7.4. When the injection of acid was begun 47 minutes had elapsed in all. During the course of the next 2 hours and 24 minutes 13.5 cc. was run in. The final hue was markedly acidotic, between *light ochraceous-salmon* and *light ochraceous-buff.* The animal died about an hour later. There was no respiratory distress at any time, in noteworthy contrast to the air hunger seen when such an experiment is done under ether.

Other similar observations were made which confirmed the findings described. When N/6.35 HCl is injected (3) the connective tissue and tendons change from yellow-pink and pinky yellow respectively to a clear orange-yellow. When the rats are stained with brom cresol purple instead of phenol red a change can be seen in the color of the cartilage, evidencing that it also has been rendered acidotic.

*The Vital Staining of Rabbits with Phenol Red.*

White rabbits can be brilliantly stained with phenol red by the injection into an ear vein of 5 cc. of a 4 per cent solution for every kilo of body weight. Within half an hour the body surface is an intense red, but soon afterwards decolorization begins, so rapidly is the dye eliminated. The animal behaves normally from first to last, and can be stained again and again without obvious injury. Most of the phthalein—as much as 96 per cent—appears in the urine within 24 hours of the injection.

The chest and abdomen of the rabbits were shaved a day or so beforehand in order to lessen the possibilities of error due to inflammation from abrasions. They were placed upon an electrically heated pad and were often covered lightly with a cloth to ensure further against an abnormal loss of body heat. The same apparatus was used to warm the injection fluid as in the case of rats but the tube leading to the vein passed directly from the water jacket to the cannula only a few centimeters away.

The color of the shaved body of the vitally stained rabbit tends to be slightly more alkaline than that of rats similarly treated. It varies with the individual from a very exceptional *jasper red* (Ridgway)[1] to *eugenia red* more commonly. The color changes can be appraised for purposes of record in terms of pH by a method already briefly described (4). An Autenrieth wedge filled with water is placed over the shaved and oiled surface of the abdomen or chest, care being taken not to exert pressure, and the color as thus viewed is compared with that noted when one or another of a series of similar wedges, filled with buffer solutions of known pH, colored with phenol red, is placed over the shaved surface of a control rabbit. By viewing through a slit the color of the various portions of the wedge placed on the control it is possible to obtain a match in color intensity as well as color character. Differences of much less than 0.1 pH can easily be read in this way. The wedges should be warmed to 35–38°C. The hue of the vitally stained but otherwise normal animal, as expressed on the pH scale, varies between pH 7.4 (*jasper red*) and pH 7.6+ (a hue slightly purpler than *eugenia red*). It will be seen that these individual variations have about the same magnitude as those which are known to occur in the normal blood. But needless to say our

[1] Ridgway's "Color standards and nomenclature" (Published by the Author, Washington, D. C., 1912) has been utilized in recording the colors, as in a previous paper of this series (Rous, P., *J. Exp. Med.*, 1926, xliv, 815). Wherever the hues provided by this book are mentioned in describing the findings italics will be employed.

use of pH figures to record the surface hue must not be taken to imply that the visible tissues have actually this pH, since there are many factors of complication. For one thing the surface is viewed by reflected light whereas readings of actual pH should be accomplished by transmitted light. The color of a fold of loose skin as seen by the latter method when it is pulled away from the body wall by gentle traction is of a different red from that observed on inspection of the same skin *in situ* by reflected light. It might be thought that the deeply stained blood would supply an important part of the surface color; but this is not the case. When all blood is washed out of the animal the surface is still an intense red (5); and whole thickness skin grafts stain heavily with the phthalein even during the period when they are completely avascular (6). One can safely conclude that the hue of the intact body surface in the living animal is referable to a distribution of the dye to the tissues; and it is upon this well justified assumption that our experiments are based. The precise whereabouts of the extravascular phthalein that renders the animal ruddy is another matter. So little of it is in the epidermis that this may be neglected; but much is in the tissue fluid,—as can be sufficiently shown by the change in color witnessed when the latter is forced out, as by compressing a skin fold between glass slides. A great deal is fixed upon the interstitial elements of the connective tissue (7). Whether the cells of this tissue contain any is doubtful.

To follow the changes in hue of the connective tissue, as distinct from those in the composite color of the body surface we have run a fine thread through the oiled skin here and there in regions where it was loose, as just above the groins; and at various periods of the experiment by pulling upon the thread have raised a fold and compressed it briefly between slides. The color of the cartilage could be followed in the ears when the circulation in them was good; but not infrequently it was so poor that the initial staining greatly lagged behind that of the body generally, as, too, did the changes consequent upon the administration of acid. In the rat the normal cartilage stains orange-yellow with phenol red, whereas in the rabbit it becomes pale pink. The more considerable alkalinity thus evidenced in the case of the latter animal is also encountered in the connective tissue which, in a compressed skin fold, appears a frank pink with only the faintest suggestion of yellow, not yellow-pink as in the rat.

By the technic described we were able to follow closely the extravascular changes in surface hue indicative of *relative* changes in pH; and, more roughly, the changes in color undergone by connective tissue and cartilage *in situ*. It remained to determine the reaction of the blood. This was accomplished by Hawkins' method (8).

The blood specimens were taken from the marginal ear vein directly into pipettes graduated to 1/100 cc., which had been ground to fit Luer needles. The latter were short, of 23 gauge, and they had been bent at a reentrant angle for con-

venience in piercing the vein in the direction of the ear tip. At the moment when this was to be done the operator made pressure upon the vein distal to the point of entry of the needle, releasing it at once thereafter so that the flow might be unimpeded. Since the vessels were distended by stroking or heat the specimens (0.25 cc.) must be thought of as consisting for the most part of arterial blood. They were procured in duplicate under oil and diluted in the usual way with salt solution. When the animal was heavily stained only enough phenol red had to be added to this solution to permit of the preliminary adjustment to pH 7.4. More of the phthalein proved necessary, of course, when the rabbit happened to be decolorizing. The protective layer of paraffin oil was replaced with melted paraffin (of low melting point) as soon as the blood and salt solution had been mixed. Readings were made in a colorimeter block at 38°C. The correspondence between the two tubes of any given specimen was, in our experience, absolute; and the whole procedure proved easy to carry out. The pipettes and needles required most careful cleansing else clotting was encountered. The same vein could be pierced again and again. This was essential to avoid using the ear into which phthalein had been injected.

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.

### *The Outlying Acidosis Attending Ether or Urethane Anesthesia.*

In initial tests the rabbits were anesthetized with ether or urethane and they lay on the back throughout the injection period. Even under the best of conditions and in the absence of anesthesia the mere weight of the body proved sufficient to give rise to an acidosis of the surface tissues pressed upon, as was shown by their change in color from red to orange; and when the limbs were stretched out under tension the circulation on the surface of the abdomen was frequently interfered with and some acidosis developed there as well. But even in the absence of these sources of disturbance there was frequently a local acidosis to be observed in the anesthetized rabbits. On injection with phenol red after they had been unconscious for some time they colored up much less promptly than did normal animals; and large irregular regions over the body and in the ears remained untinted after the red staining had become pronounced everywhere else. When phthalein did at length penetrate into these regions their color had an orange cast. Evidently a local acidosis had developed in them, secondarily to an interference with the blood supply. As

is well known a blood acidosis develops during ether anesthesia. But independently of any such change, there was an extravascular acidosis, an "outlying acidosis" resembling that we have described in other connections (9).

*Experiment IV.—Effects of urethane.* 142 cc. of N/6.35 HCl was run into the jugular vein of a male white rabbit (No. 1) weighing 1625 gm., which had been given 3 cc. of 50 per cent urethane into the peritoneal cavity 2 hours beforehand. The injection took 1 hour and 40 minutes. When 50 cc. had been given 10 cc. of 4 per cent phenol red isotonic with 0.9 NaCl and at pH 5.6 was slowly introduced into an ear vein, without intermitting the flow of acid to the jugular. The animal stained only very gradually and irregularly *jasper red*, with patches coloring up later of *coral red* here and there. As time passed and the HCl injection proceeded the color altered more and more toward orange, and had become *rufous* in some places, *apricot orange* in others, by the time all the acid was in. The determinations on the blood showed a fall in pH from 7.4 prior to the giving of the acid to 7.0 immediately afterwards. Later the surface color became still yellower, between *zinc orange* and *ochraceous-salmon*, instead of altering toward red, and the animal died 1 hour and 40 minutes after the injection had been finished. From first to last there was no air hunger despite the acidosis. The surface hue at the end corresponded with pH 6.8.

In this instance not only was the staining irregular, with relatively acidotic areas here and there, but the complete absence of a compensatory increase in the breathing attested to a depressant effect of the urethane. To the absence of this respiratory compensation death was attributable, the animal becoming more and more acidotic after the injection was stopped, instead of tending to recover as happens after ether or in the absence of any general anesthesia. Cushny and Lieb have shown that the reaction of the respiratory center is altered in rabbits deeply under urethane (10).

*Experiment V.—Effects of ether.* The rabbit (No. 3) weighed 1600 gm. The *first blood specimens*, taken just prior to a light etherization, were at pH 7.4+. Now the jugular vein was cannulated, and 35 minutes after the blood had been taken the acid injection was begun. When 71 cc. of N/6.35 HCl had been run in the cannula was flushed with salt solution by means of a three-way stop-cock connecting with it, and 10 cc. of 4 per cent phenol red isotonic with 0.9 NaCl and at pH 7.4 was gradually injected. The animal colored only very gradually, *jasper red* here, *coral red* there, with a streak of orange-yellow along the midline from ensiform to symphysis. As more and more acid was injected the respirations became stormy and the general color changed to *apricot orange* variegated with

*rufous*. After 160 cc., given in the course of 2 hours, the jugular was tied and the ether, which for some time had been almost left off, was wholly discontinued. The *second blood specimens* were now taken, reading midway between pH 6.8 and 6.9. The *surface hue* on the other hand corresponded with 7.0− to 7.0+. The animal soon got to its feet and shortly thereafter the staining lost its patchy character. During the rest of the day it appeared in good condition save for the exaggerated respirations. Several blood specimens were taken. These showed only a slight return toward a normal reaction whereas there was a more marked extravascular return as shown by the surface color. The changes find record in the chart (Chart 1).

The animal was given food and left for the night. Next morning it was reported to be decolorized and in good condition, having eaten well. Unfortunately it was placed in a cage with other animals to be brought to the laboratory, and, as it moved about amongst them, it suddenly collapsed and died, making violent respiratory efforts. Apparently its slight exertions had been sufficient to overturn the *status quo*. At autopsy no anatomical cause for death was found.

In this experiment (Chart 1), which was duplicated by another, the injection of an enormous amount of the acid solution was withstood by the animal. There was the stormy respiration usual in acidosis, a feature which had been lacking with urethane, and after the injection the animal tended to recover as had not been the case when the latter was used. The staining with phenol red, done at a time when considerable acid had been run in, disclosed a poor circulatory condition of the tissues, as shown by a tardy distribution of the dye and the presence of relatively acidotic areas over chest and abdomen. The rapid change to an even staining after the animal had come out of the anesthetic was a significant feature as was also the return of the surface color toward the normal during a period when the blood reaction remained almost unchanged. As the later work showed, this return was referable to the disappearance, as the circulation became better, of a generalized "outlying acidosis" which had been present in addition to the extravascular acidosis induced by the HCl. In none of the later experiments, in which a local anesthesia took the place of a general one, was any pronounced color patching noted like that with ether or urethane; in none did the hue become as orange,—despite the fact that the injection of acid was pushed to the extreme; and in none did the extravascular reaction return more rapidly to the normal than that of the blood.

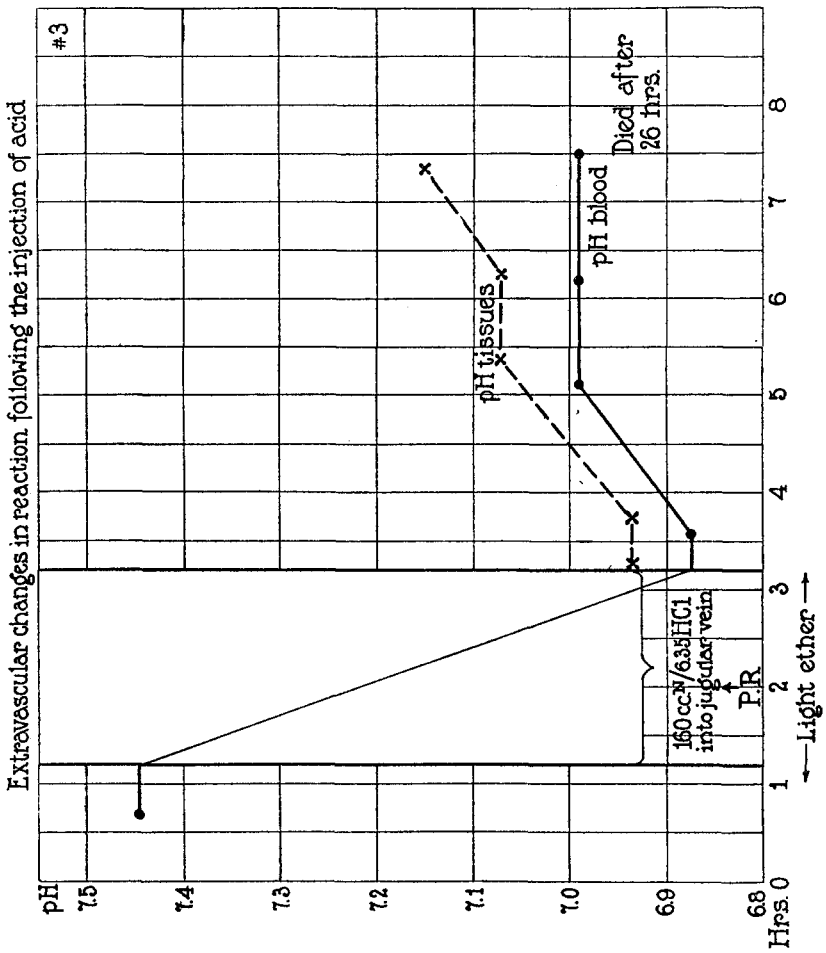
Extravascular changes in reaction following the injection of acid
#3
pH
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
Hrs. 0
1
2
3
4
5
6
7
8
←Light ether→
160 cc N/6.35 HCl into jugular vein
P.R.
pH tissues
pH blood
Died after 26 hrs.

CHART 1.

For the purpose of the foregoing observations the animals were stained with phenol red only after the injection of acid had proceeded for some time; since otherwise the staining did not last long enough to cover the entire later period of study. The plan was now adopted of giving smaller amounts of the indicator with repetitions of the dose at more frequent intervals. Tests directed to the point had shown that the intravenous injection of 10 cc. of 4 per cent phenol red, brought to pH 7.4, exerted not the slightest effect on the reaction of the blood. To expose the jugular vein novocaine was employed, without precautions for sterility. After the injection was finished the neck incision was sutured and the animal placed by itself in a metabolism cage where it could be carefully watched. Abstracts of two protocols will be given.

*Experiment VI.*—(See Chart 2.) Male white rabbit (No. 6) of 1690 gm. 9.30 a.m.—*First blood specimens taken.* 9.37—First injection of phenol red. 10.05—Rabbit coloring rapidly and evenly. 10.22.—*Surface hue* is *old rose;* by wedge method pH 7.5. 10.30—*Second blood specimens taken.* 11.29—*Injection of* N/*6.35 HCl begun* at rate of 5 cc. every 3 minutes. 12.06 p.m.—65 cc. in. Animal completely decolorized. 12.35—102 cc. in. Pause for injection of phenol red, 10 cc., by way of the jugular cannula, between 12.36 and 12.39 p.m. Injection of acid now renewed at a slower rate. 12.56—115 cc. of acid has been injected. The animal is everywhere *jasper red.* 2.29—The general color is *coral red* but with carroty areas here and there. *Injection terminated and animal removed from pad.* It has received 170 cc. of acid in all. 2.50—*Third blood specimens taken.* Animal O. K. 3.00—*Surface hue* between *coral red* and *carnelian red;* by wedge method pH 7.2. 4.33—*Fourth blood specimens taken.* 4.39-40—Injection of phenol red, 5 cc., into an ear vein. 5.13—*Surface hue* by wedge method pH 7.3−. 5.18—*Fifth blood specimens taken.* 5.41—*Surface hue* by wedge method just about pH 7.3 (artificial light).

During the latter part of the period of injection the respiratory amplitude was greatly increased. The animal remained quiet throughout, however. Afterwards it voided large quantities of urine colored orange-red with phthalein. During the night it escaped from the metabolism cage, and some urine may have been lost. Next morning the respirations were still as exaggerated as on the previous evening, and the animal was weak and apathetic.

*Second Day,* 9.55 a.m.—*Sixth blood specimen taken.* 10.08—Injection of phenol red, 5 cc. into an ear vein. 10.20—The rabbit has colored but poorly, yet there is no evident outlying acidosis. 10.39—The coloration is diffuse; *surface hue* by wedge method, pH 7.3+. 11.18—*Seventh blood specimen taken.* 11.33—*Surface hue* by wedge method, pH 7.3. Respirations still exaggerated. 12.07—There is

a sudden commotion in the cage, and the rabbit previously quiet, is found in opisthotonos. It died at 12.10.

The autopsy showed atelectasis of the upper lobes of both lungs, and in the right psoas muscle a contracted blood clot apparently about 24 hours old with a volume of about 10 cc.

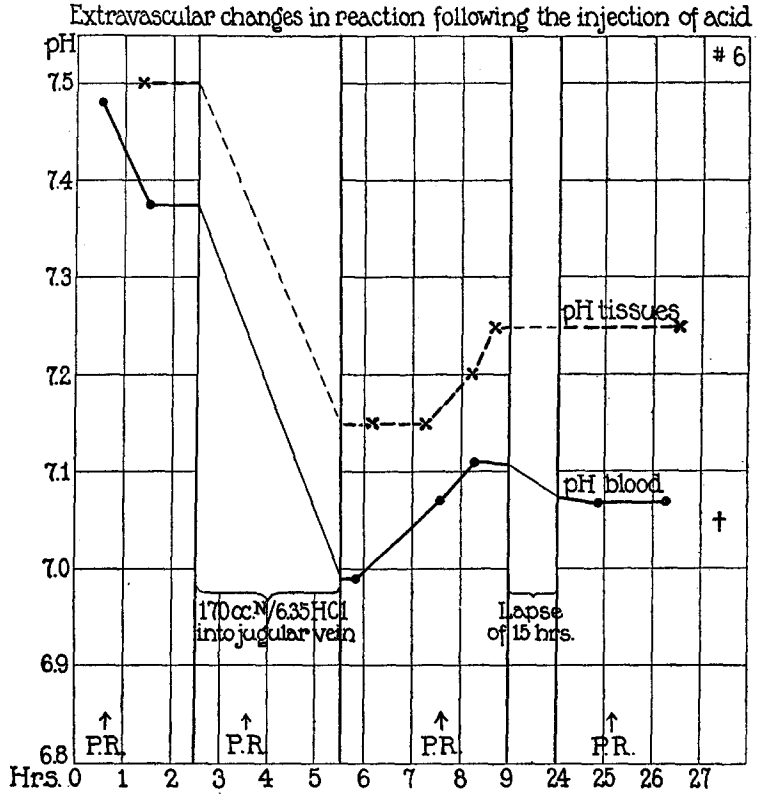


CHART 2.

The bladder had been emptied by catheterization at the beginning of the experiment. From then until death 184 cc. of urine was secreted, an amount somewhat larger than the total of acid solution injected. The rabbit had free access to water during the night and to food but appeared not to have partaken of either. The amount of circulating hemoglobin fell from 78 per cent to 58 per cent (Newcomer), and the red cells from 6,590,000 to 3,490,000 per c.mm. in the first 24 hours. A part of the blood change is attributable to the hemorrhage into the muscle.

In this experiment the maximum quantity of acid was given that could be borne. The reaction of the blood was altered from pH 7.4 to pH 7.0, and during the next 24 hours it scarcely changed (Chart 2). The surface color, recorded in terms of pH, gave readings that were regularly further on the alkaline side than those of the blood; but, as has already been emphasized, these readings cannot under the conditions be taken as expressive of the actual pH prevailing. They have merely a relative value as indicative of the extravascular condition. The change in this would appear to have been not quite so great as in the blood, the apparent pH falling from about pH 7.5 to pH 7.2. The later, slight recovery in the blood was duplicated, however, by an extravascular recovery of equal magnitude.

The death of the animal cannot be attributed directly to the acidosis because of the lesions found at autopsy. The question comes up, indeed, as to whether the persistence of the acidosis may not have been due to these latter. That it was not, the following experiment shows.

*Experiment VII.*—Male rabbit (No. 7) of 1650 gm. Jugular bared under local anesthesia with novocaine. 9.42—*First blood specimens taken.* 9.50½-52—Injection into an ear vein of 4½ cc. phenol red. 10.22—*Surface hue* between *old rose* and *eugenia red;* by wedge method, pH 7.5. 10.53—*Second blood specimens taken.* 11.02—*Surface hue* by wedge method pH 7.5. 11.46—*Injection of* N/*6.35 HCl begun.* 12.36 p.m.—59 cc. has been injected. 1.13—Injection of 5 cc. phenol red by way of the cannula in the jugular. The animal colored evenly and quickly, *jasper red.* 1.46 p.m.—132 cc. of acid in. *Surface hue* between *light jasper red* and *coral red.* 2.02 p.m.—142 cc. of acid in. Rabbit is restless; breathing greatly exaggerated. 2.19—Animal nearly decolorized. 2.24 p.m.—149 cc. in. 2.24-26½—10 cc. of phenol red injected into jugular. A rapid and deep staining developed. 3.01 p.m.—158½ cc. of acid has been run in; *injection terminated* and incision sewed up. 3.10—*Surface hue* between *coral red* and *jasper red.* 3.11—Rabbit removed from board. It is alert but stands weakly, and has a notable air hunger. 3.26—In excellent condition, though still weak and with air hunger. *Surface hue* now slightly darker than *carrot red.* 3.43—*Surface hue* by wedge method between pH 7.1 and pH 7.2. 3.52—*Third blood specimens taken.* 4.00—*Surface hue* again between pH 7.1 and pH 7.2. 4.51—*Surface hue* just above pH 7.1. 4.57—*Fourth blood specimens taken.* 5.03—*Surface hue* between pH 7.1 and 7.2. 5.20—Decolorizing. Left overnight, with water but no food. Eager to eat cabbage shown it.

*Second Day.* 9.35 a.m.—Animal in excellent condition except for weakness of fore leg on side of jugular injection. Breathing still exaggerated. 9.44—*Fifth*

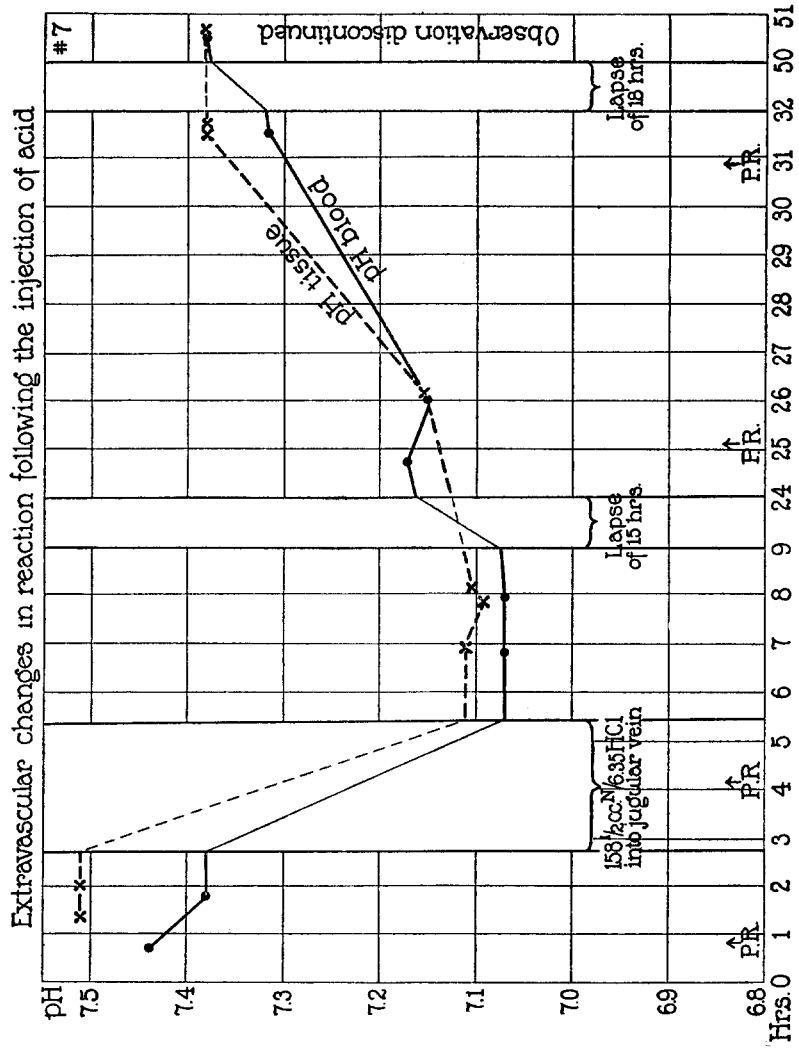
Extravascular changes in reaction following the injection of acid
#7
Observation discontinued
pH tissue
pH blood
Lapse of 18 hrs.
Lapse of 15 hrs.
15 cc. N/6.35 HCl into jugular vein
P.R.
pH
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
Hrs. 0 1 2 3 4 5 6 7 8 9 24 25 26 27 28 29 30 31 32 50 51

CHART 3.

*blood specimens taken.* 10.04-06—7 cc. of phenol red injected into an ear vein. The staining that followed was rapid, even, and deep; *surface hue* by wedge method pH 7.3. 10.41—*Surface hue,* by wedge, just below pH 7.3. 11.00—*Sixth blood specimens taken.* 11.04—*Surface hue* by wedge, pH 7.2. 11.09—Color is between *light jasper red* and *light coral red,* slightly darker than either. 11.32—Given cabbage which it eagerly attacks. 2.15—Has eaten largely; condition excellent though right fore leg is still dragged. 3.53—Injected with 7 cc. of phenol red. 4.14—General color *jasper red.* 4.32—*Surface hue* by wedge, pH 7.4. 4.35—*Seventh blood specimens taken.* Still some air hunger on exertion. 4.42—*Surface hue* pH 7.4. 6.13—Left overnight with food. General condition as before.

*Third Day.* 10.24 a.m.—Has again eaten largely. Injected with 7 cc. phenol red. 10.35—Given more cabbage; eating. 11.07—*Surface hue* by wedge pH 7.4, and again pH 7.4 at 11.24. 11.28—*Eighth blood specimens taken. Surface hue* by wedge pH 7.4. Decolorizing; color is *old rose.* 12.27—Not yet fully decolorized; observations terminated.

*Fourth Day.* Weight 1500 gm.

*Fifth Day.* The tissues of the neck are indurated about the old wound. Animal killed with chloroform. The general condition of the organs is excellent save in in the case of the kidneys, the renal cortex appearing somewhat swollen and paler than normal (parenchymatous degeneration?).

The bladder had been emptied by catheterization prior to the injection of acid, and during the 19 hours thereafter 223 cc. of urine heavily colored with phthalein was voided. During this period no food had been given.

The hemoglobin dropped from 75 per cent prior to the experiment to 58 per cent on the next morning, and the number of red cells from 5,460,000 to 4,430,000. In the afternoon the hemoglobin percentage was 65 and the number of red cells 4,750,000. On the 2nd day the figures were the same. For the determinations and those of Experiment VI we are indebted to Dr. Charles A. Doan.

In this experiment (Chart 3) the alteration in the surface color, as recorded in terms of pH, was slightly greater than that found in the blood, whereas in the preceding one it had been slightly less. The correspondence between the later extravascular changes, indicated by the surface hue, and the intravascular ones was exceedingly close. The observer following the alterations in the blood did not know of those in the surface hue. The variations in pH were almost identically the same in both, and the same very gradual recovery took place until food (cabbage) was given, when the acidosis rapidly lessened, almost disappearing within a few hours. How long recovery might have taken had no materials to combat the acidosis been supplied by mouth is problematic.

The amount of urine voided by the animal during the first 19 hours after the acid injection (223 cc.) considerably exceeded the fluid bulk introduced in this way (158½ cc.).

The diminution in the amount of blood in circulation attested by the lessening in hemoglobin and in the number of circulating red cells is to be attributed in no small part to the repeated bleedings incident to the observations on the blood reaction. The remaining loss was doubtless due to an action of the hydrochloric acid solution on such red cells as came into direct contact with it during the injection period.

*Significance of the Extravascular Changes.*

The alterations in the surface color that took place during the foregoing experiments might be attributed wholly to changes in the reaction of the stained extravascular fluid did not incidental observations show that the hue of the tissues themselves was altered. We have already described the method whereby it is possible to determine the hue of connective tissue *in situ*. Oiled folds of skin of the vitally stained rabbit when viewed between slides by transmitted light are ordinarily pink even when the fluid has been driven out of them by pressure upon the slides. After the injection of acid they appeared orange-pink until pressure was made when they were seen to be of a clear orange-yellow, far more acid, judging from the color, than the body surface as viewed by reflected light. The conjunctiva likewise became orange, and the sclera palely so. The cartilage in the least vascular portions of the ears of vitally stained rabbits is pink ordinarily. But after the acid injection it was a clear orange-yellow. In proportion as the general acidosis was recovered from, so too did all of the tissues mentioned alter from orange to pink. On the inside of the ear where the cartilage is covered with merely a film of adherent skin its color can be readily followed by reflected light; and the observation was made that at times when the alteration in the hue of the body surface generally had progressed no further than *coral red* the cartilage had become a clear orange. The finding would suggest that despite the relative alkalinity of normal rabbit cartilage as compared with that of the rat or mouse, an alkalinity finding expression in a pink staining with phenol red,

this tissue is still not so alkaline as the connective tissue, which in turn is less alkaline than the blood (11). The pH of normal rabbit blood is almost identical with that of the rat (12).

## DISCUSSION.

The findings show that when changes in the reaction of rabbit blood are induced with hydrochloric acid extravascular changes of approximately the same magnitude occur. No dissections were made to inspect the interior organs; but from previous experiments on rats it seems certain that conditions of blood acidosis or alkalosis leading to a change in the color of connective tissue and cartilage will also lead to one in the color of the tendons, whereas the hue of liver, pancreas, and lymph nodes will be unaffected even when the blood change is so great as to lead to death (13). There is every reason to suppose that the same holds true in the case of rabbits, and that by following the visible alterations in the connective tissue and cartilage one can obtain an index to much of what would be disclosed by dissection under oil.

The failure of the extravascular reaction to alter more than does that of the blood would at first sight seem surprising. For, with a lessened gradient between tissues and blood, one might suppose that there would be some heaping up of the acid products of metabolic activity. Perhaps such a heaping up and not a passage of H ions from the blood is actually one of the causes of the extravascular acidosis. Whenever the circulation is interfered with, as during ether and urethane anesthesia, a heaping up of acid metabolites certainly does occur, with result in an "outlying acidosis" independent of that due to the injected acid. One is reminded in this connection of Yandell Henderson's statement that most patients and animals under general anesthesia for more than an hour are in the first stage of shock (14). Our experiments prove that under the conditions of such anesthesia an abnormal local reaction develops within some of the tissues which may well prove detrimental to the organism as a whole.

The demonstration that a widespread extravascular acidosis occurs when there is a marked blood acidosis, and that it is tolerated for

long periods has a bearing upon the problem of shock. For many years now the relationship between the functional state of the small blood vessels and the reaction of their milieu has been debated; and many have supposed that a vascular dilatation is caused by acidosis and that such a dilatation may be responsible for shock through the changes in the distribution of blood that it entails. The British Commission for the investigation of surgical shock could not produce it by injecting acid into the circulation (15); but they were unable to bring proof that in altering the condition of the blood they also altered the reaction outside of the lumen of the vessels. That extravascular changes go hand in hand with those taking place in the blood has been demonstrated in the course of the present work. Yet not the least evidence of shock was to be seen in our animals though the blood reaction was diminished to the limit compatible with life and the extravascular reaction of large regions was proportionately affected. Though the connective tissue was markedly acidotic, as shown by its color during the periods of staining, not the least vascular dilatation could be observed in it through the shaved and oiled skin. In the intervals of decolorization between the injections of phenol red there was good opportunity to make observations on the latter point.

The quantities of fluid voided by our animals during the many hours that the tissue acidosis endured regularly exceeded the very large amounts introduced with the acid. These animals had access to water but were given no food, and they certainly did not drink largely. There was not the least sign of a fluid retention in the acidotic tissues. In a previous paper (16) one of us has demonstrated that local acidosis and edema sometimes occur together but without any relationship of cause and effect. The present findings afford another example of the phenomenon.

## SUMMARY.

The changes in blood reaction caused by the injection into a vein of a weak solution of hydrochloric acid are accompanied by extravascular changes of similar magnitude within the subcutaneous tissue. Under the conditions of prolonged general anesthesia with

ether or urethane the circulation to this tissue is so interfered with that an "outlying acidosis" may develop in addition to the acidosis immediately consequent on the blood state. Even under the best of circumstances the extravascular acidosis induced with hydrochloric acid affects not merely the tissue fluid but the reaction of the tissue itself.

Rabbits in which a widespread extravascular acidosis has been produced, together with a blood acidosis as severe as is compatible with life, remain in good condition during the relatively long period over which this state of affairs persists. There is at no time any sign of capillary dilatation, though the vessels are bathed in relatively acid fluid, and none of shock. No edema develops in the acidotic tissues, and the animals void large amounts of urine. The tissue acidosis lessens *pari passu* with that of the blood.

BIBLIOGRAPHY.

1. Rous, P., and Beattie, W. W., *J. Exp. Med.*, 1926, xliv, 835.
2. Rous, P., and Beattie, W. W., *J. Exp. Med.*, 1926, xliv, 835.
3. Rous, P., and Beattie, W. W., *J. Exp. Med.*, 1926, xliv, 835.
4. Rous, P., and Beattie, W. W., *J. Exp. Med.*, 1926, xliv, 835.
5. Rous, P., *J. Exp. Med.*, 1925, xli, 451.
6. Rous, P., *J. Exp. Med.*, 1926, xliv, 815.
7. Drury, D. R., and Rous, P., *J. Exp. Med.*, 1926, xliii, 669, 687.
8. Hawkins, J. A., *J. Biol. Chem.*, 1923, lvii, 493.
9. Rous, P., and Drury, D. R., *J. Am. Med. Assn.*, 1925, lxxxv, 33.
10. Cushny, A. R., and Lieb, C. C., *J. Pharmacol. and Exp. Therap.*, 1914–15, vi, 451.
11. Rous, P., *J. Exp. Med.*, 1925, xli, 739.
12. Hawkins, J. A., *J. Biol. Chem.*, 1924, lxi, 147.
13. Rous, P., and Beattie, W. W., *J. Exp. Med.*, 1926, xliv, 835.
14. Henderson, Y., *Physiol. Rev.*, 1923, iii, 165.
15. Cannon, W. B., Cowell, E. M., Fraser, J., and Hooper, A. N., Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions, No. 2, Great Britain Medical Research Committee, December, 1917.
16. Rous, P., *J. Exp. Med.*, 1926, xliv, 815.