# OUTLYING ACIDOSIS DUE TO FUNCTIONAL ISCHEMIA.

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(Received for publication, October 16, 1928.)

In a note from this laboratory published some little time ago (1) the occurrence was reported of states in which a frank acidosis of the tissues develops without any change in the reaction of the blood. The purpose of the present paper is to describe more fully this outlying acidosis and to consider its causes.

Most of the blood acidoses encountered by the clinician arise through tissue activities, needless to say—those referable to acid materials taken by mouth being highly exceptional. Inevitably the aim of students of acidosis must be to determine the where and the how of its production by the organs. Despite the perfecting of methods for the study of blood states and of the excreta we are still, in respect to this problem, much like explorers unable to penetrate into a continent, and gaining information of the life there only by the observation of what is borne on and out by its great rivers.

## *Local Acidosis Due to Mechanical Interference with the Circulation.*

The fact that under circumstances of deficient oxidation tissues form acid substances has long been commonplace. One would expect that where the circulation is seriously impaired both these and the acid products of ordinary cell activities will tend to be retained. Experiments upon animals vitally stained with indicators have demonstrated that the accumulation under such circumstances is frequently sufficient to affect the tissue reaction perceptibly. Vigorous skin grafts are relatively acid during the days before they become vascularized (2). In a normal rat vitally stained with phenol red interruption of the circulation to a leg by means of a tourniquet leads within a few minutes to a change in the color of the limb from red to orange-yellow. These are simple instances of acidosis in the superficial

tissues. We have carried out ligation experiments to determine whether evidence can be had elsewhere of a similar state of affairs.

*Experiment I. The Local Manifestations of Acidosis on Direct Interference with the Circulation.*—White rats were employed which had been shaved where necessary for the observations and stained with phenol red or brom cresol purple. The methods of staining by intraperitoneal injection, and of inspection have been fully described in previous papers (3–5). The shaving had been skilfully done some days beforehand. In order to minimize the loss of carbon dioxide from organs exposed during the course of the work the animals were submerged in paraffin oil kept at body temperature (5). Sections of the parenchymal tissues were made under oil with the Valentine knife, normal portions of the same organ, or of its fellow in the case of paired organs, serving as control. They were examined immediately, between mica slides.

In brief it was found that when the circulation to an extremity had been interrupted the reaction of the subcutaneous tissue undergoes a manifest alteration toward acidity within a few minutes, the alteration being sufficient to change the color, not merely of phenol red, but of brom cresol purple (from purply blue to blue-green). A similar change of brom cresol purple occurs even more rapidly in the skeletal muscles. In them, though, the alteration is traceable to that part of the phthalein which is held in the tissue fluids, a fact readily to be demonstrated in the long muscles of the leg. The belly of one of these muscles in which the circulation has been stopped for some minutes appears greenish purple when viewed *in situ* with a piece of white card slipped under it as background, whereas the control muscle in the unaffected leg is clear purple. But if the two are gently compressed between glass slides to drive fluid out of them they take on almost precisely the same purply green hue. This might have been predicted; for the muscle fibers themselves stain a light greenish yellow with brom cresol purple even under ordinary circumstances (4), a hue so far toward the acid end of the indicator range that any change toward acidity which might occur in such fibers would be unaccompanied by any pronounced change in the general color,—this last being, of course, composite in make-up.

The color change occurring in portions of the spleen with ligated vessels is likewise confined almost wholly to the phthalein dissolved in the tissue fluids. The organ stains but poorly with the indicator.

The tissues of liver and kidney do not become evidently more acid to brom cresol purple until 15 or 20 minutes after the circulation has been stopped; but the reaction of their parenchyma is then definitely affected as shown both by observation under the microscope and by the pressure test. The small intestine becomes acid somewhat more rapidly. On the other hand, the reaction of the tendons, and of fascia at a distance from muscle, as for example in the lower leg or tail, changes not at all during a half-hour or more of circulatory obstruction.

These observations, while fragmentary, suffice to bring out significant differences in the degree of color change, as indicative of acidosis, in various organs deprived of their circulation. The fact that no alteration occurs for a long time in tendon and fascia is in keeping with what is known of the metabolic rate of these tissues. On the basis of relative rates a much more prompt alteration had been expected in the kidney and liver than in the subcutaneous tissue. But as a matter of fact a change was regularly evident within this tissue after only about a third of the time required for the development of one in the parenchymal organs mentioned. The fact may be recalled in this connection that the injection of acid or alkali into the circulation fails to alter the reaction of the liver, even when the procedure is pushed to the limit of tolerance and the reaction of the blood and matrix tissues (tendon, connective tissue, cartilage) has been pronouncedly altered (5). Very probably the hepatic cells, and quite possibly cells in general, maintain their reaction so long as they are uninjured, the acid products of their metabolism being cast out into the surrounding fluid or segregated within the protoplasm.

Because of the difficulties of interpretation brought out by the foregoing experiment, as well as for obvious reasons of expediency, it seemed best to begin the study of outlying acidosis with observations on the superficial connective tissue.

### *Local Acidosis Due to Epinephrin Ischemia.*

A type example of local acidosis from functional ischemia, that which follows upon the subcutaneous injection of epinephrin, has been investigated at length.

*Experiment II. The Local Changes in Reaction Caused by Epinephrin.*—As in the previous experiment, white rats were used, vitally stained by the injection of phenol red or brom cresol purple into the peritoneal cavity. Sometimes the animals were anesthetized by the injection of a urethane solution into the tissues of the back of the neck (5). In such case they were laid upon an electrically warmed pad and the shaved body surface was swabbed with paraffin oil, a procedure which brings out brilliantly the hue of the stained tissues. Several specimens of 1:1000 adrenalin chloride (Parke, Davis and Company) were employed. These proved acid to phenol red, and were brought to pH 7.4 just prior to use by titration with N/10 NaOH in the presence of a trace of the indicator. Control tests on stained animals demonstrated that the amount of chloretone present in the epinephrin preparations had no effect to alter the local tissue reaction.

A fraction of a drop of the epinephrin solution introduced through a fine needle into the subcutaneous abdominal tissue of unstained rats, well to one side of the midline, caused blanching along the needle track in 7 to 10 seconds, and this blanching spread until, within the course of a few minutes, there existed a pallid patch 2 or more cm. broad. In animals stained with phenol red on the other hand, no swift blanching or other color change occurred along the needle track,—fresh evidence in addition to that already provided (3) that the staining is essentially extravascular. After about 2 minutes, however, an alteration from red toward yellow could be noted at the site of injection; and within 5 minutes there was present a well demarcated orange patch of the same dimensions as the blanched patch to be seen at this time in unstained animals. During the next half-hour this patch persisted and often spread somewhat, the color becoming a marked orange-yellow. Meantime the staining of the animal as a whole frequently became less intense, owing to elimination of the dye; but this process failed to affect the color of the patch which in consequence soon came to stand out boldly on a pink background.

The first sign of a wearing off of the effect of the adrenalin was a reversion from orange to red at the margin of the patch. Occasionally a rosy spot developed at its center as well. When the newly reddened tissue was compressed between slides its aspect did not alter immediately,—again showing the extravascular situation of the dye,—but after a few minutes of such compression it turned yellow, becoming red once more when released. These changes were very vivid when they occurred, as not infrequently, in rats that had decolorized everywhere else. Under these circumstances the marginal tissue after turning red soon decolorized too, and the patch dwindled in size by a peripheral reversion from yellow to red, followed by removal of the dye. The time from epinephrin injection to complete disappearance of the patch was usually almost 2 hours.

That ischemia was the cause of the local acidosis responsible for the color change became clearly evident when the epinephrin injection preceded that of the phenol red by 5 minutes or more. Then one saw, as the animal became vitally stained, that none of the dye entered the areas blanched by the epinephrin, the result being that they stood out as pallid blotches against surroundings that had rapidly colored an intense red with the highly diffusible phthalein. For as long as a half-hour this phenomenon persisted, and then, as the adrenalin effect wore off, color crept in gradually at the edges and sometimes at the center of the pale blotches; and where this took place the tissue was at first stained orange-yellow, turning orange later and then red.

Brom thymol blue could not be utilized in the attempt to gauge the acidity of the ischemic patches because it is not only toxic but gives false indications *in vivo* (4, 6); and no other indicator with its range has been found satisfactory. Evidence corroboratory to that with phenol red could, however, be obtained by vital staining with brom cresol purple. The subcutaneous tissue of the rat has a reaction which causes it to take on a hue about midway in the rather wide and but slightly various purple portion of the range of the indicator (5). For this reason

considerable alterations in pH are required to bring about a manifest color change *in vivo*. Yet such a change was sometimes apparent in subcutaneous regions rendered ischemic by adrenalin,—one from frank purple to greenish purple. A similar but more pronounced change was visible in the ear of the stained rat, injected with adrenalin and viewed by transmitted light. In the subcutaneous tissue of the abdomen N/100 HCl gave rise to a color change about as great as that due to epinephrin; but N/10 HCl turned the purple to yellow. In animals stained with phenol red the epinephrin patches were never so yellow as those developing elsewhere in the same animals after subcutaneous injection of N/10 HCl. The acidity in the ischemic patches was, in other words, never so great as to lie entirely beyond the range of phenol red, a finding confirmed by the observations with brom cresol purple.

Some specimens of epinephrin caused a greater color change in animals stained with phenol red than did others, whence one may perhaps infer that they had a more intense vasoconstrictor action.

The fact is plain from these observations that temporary ischemia due to a local contraction of the subcutaneous vessels leads within a few minutes to acidosis in the region affected. Much of this can be referred to the accumulation of carbon dioxide,—a factor dealt with in previous papers from this laboratory (2, 3). When the skin is everted, ventilating the patches, their color quickly changes to red and then to purply rose. Carbon dioxide passes through the tissues with such ease (7), and in the rat the tissue layer upon which the epinephrine acts is so shallow,—even when, as sometimes happens, the underlying muscle partakes of the ischemia,—that a not inconsiderable escape of the gas from the patches into their surroundings must be supposed to occur even when the circulation is at a standstill in them. This would tend to mitigate the acidity.

It is possible to gauge with considerable accuracy the apparent pH of superficial tissues stained with phenol red and to measure the extent of the changes taking place in the reaction (8). But that was not attempted in these experiments.

### *Paradoxical Acidosis.*

The foregoing observations justify the belief that in clinical conditions which involve a functional impairment of the circulation to special regions, as *e.g.* in Raynaud's disease, a local acidosis may develop so considerable as to alter the tissue reaction. The occurrence of widely distributed changes in the tissue reaction independent

of blood changes was brought to our attention by a singular and convincing instance. A shaved white rat stained with phenol red had been given several cc. of a strong solution of sodium carbonate by stomach tube. The blood reaction became more alkaline soon after, and alkaline urine was voided; yet the shaved body instead of participating in the alkalinity, as shown by a change in hue of the stained tissue from red to purply rose, turned orange-yellow, became relatively acid that is to say. The phenomenon can be elicited at will.

*Experiment III. Outlying Acidosis Caused by Sodium Carbonate.*—Shaved rats given no food for 24 hours but allowed water until the time of the experiment were given an intraperitoneal injection of isotonic 4 per cent phenol red at pH 7.4; and, when they were well stained, warmed N/1 sodium carbonate (Merck) was administered by stomach tube in amounts of from 3½ to 10 cc., depending upon the weight of the animal and how extreme were the instances desired. Amounts greater than 5 cc. were given in divided doses since otherwise regurgitation often ensued. The pH of the blood was followed by the aspiration of samples from the heart into short segments of ordinary 1 cc. pipettes graduated in hundredths, which had been ground to fit short aspirating needles of stainless steel. 0.2 cc. of blood was taken at a time, a paraffin seal preventing the entrance of air at the junction of glass and needle during the aspiration. The pH was determined by a modification of Hawkins' method (9), whereby 0.1 cc. of blood was introduced into 2 cc. of salt solution previously tinted with phenol red and brought to pH 7.4 under oil. Mixing was accomplished with a thin glass stirring rod, the oil quickly replaced with paraffin that solidified on cooling, the tube centrifuged, and the color read at 38°C. in an ordinary comparator block. Many tests in duplicate showed that the findings with a single one could be relied upon, as also that the pH of the blood-salt mixture did not change during a period much longer than that which elapsed prior to the readings. The remainder of the blood specimen was utilized for determination of the cell-plasma relationship by Van Allen's method (10) which requires only a fraction of a drop. Here too control tests demonstrated the validity of our technic. It seemed better to repeat the experiment often than to take each time the amount of blood that would have been required for duplicate tests.

The first blood specimen was withdrawn after the vital staining was well established, and thereafter the animal was watched for signs of heart injury during some minutes before the carbonate was given. Usually observations were carried out on several animals at a time, kept on absorbent cotton in separate jars. The blood pH of controls merely stained with phenol red did not vary, nor did the surface color alter.

Unanesthetized rats of about 150 gm., receiving 5 to 7½ cc. of N/1 sodium carbonate, appear uncomfortable within 15 minutes, the hair roughening; and soon they begin to jump intermittently and aimlessly. After 20 minutes to ½ hour in

typical instances the color of the shaved and oiled belly is noted to be changing from red toward yellow. At this time the animal is still on its feet and appears strong. It is now often voiding urine that is alkaline, as shown by the hue of the phenol red it contains, whereas that passed prior to the carbonate was acid by the same criterion. Within an hour the animal is cold and feeble, sitting quiet, or in a state of semicollapse; and its color is an outspoken orange-yellow. Often it dies a little later, with the yellow hue even more pronounced. If it is to survive, an active purgation of semifluid, rose-purple material takes place, together with an alkaline diuresis, and decolorization is complete in the course of 3 or 4 hours. Not infrequently, when only 5 cc. of carbonate is given, no signs of acidosis develop. In our animals that succumbed after administration of the salt the heart was examined for injury from the aspirating needle, and instances in which this might have caused death were ruled from consideration.

The second blood specimen was ordinarily taken when the surface of the body appeared notably orange. There was either no change in pH or else a rise (from pH 7.4–5 to pH 7.6 or even to pH 7.7 in some cases).

To study carefully the progress of the acidosis numerous rats were urethanized, and after the carbonate had been given they were placed in the oil bath (5). In this way the influence upon the phenomenon of chilling of the shaved skin was ruled out. Urethane of itself has no effect upon the pH of rat blood within periods so short as those of our experiments (11). In rats under its influence the blood specimens were taken directly from the common carotid artery. The vessel was dissected free, seized with a mosquito forceps, and the wound cavity filled with oil. The forceps was given a quarter turn around its axis and away from the heart so that the distended vessel lay in a curve over its blades and the needle was inserted at the curve and toward the heart. As the needle was withdrawn, after 0.2 cc. of blood had been obtained, the flow was checked either by turning the clamp a little further, or preferably by traction upon a thread placed loosely about the vessel. A fine ligature was then put on at leisure. It was found that no blood need be lost by the method and that the same vessel can be aspirated several times. The needle employed should have a short bevel, and should be inserted with the bevel side up.

The findings in urethanized rats confirmed those in the unanesthetized; and much less carbonate sufficed to bring about the acidosis.

### *Relation of Anhydremia to the Acidosis.*

The development of tissue acidosis in the rats given sodium carbonate was regularly accompanied by a change in the cell-plasma relationship of the blood, the proportion of cells increasing markedly, sometimes enormously, at the expense of the plasma (from 43.5 to 64.5 per cent; from 44 to 67 per cent,—to cite but two instances out of a large number). The degree of the acidosis was paralleled by

that of the blood change. That this change was the expression of an anhydremia cannot be doubted; for not only was the N/1 sodium carbonate solution strongly hypertonic, but the amount of fluid accumulating out of the body into the bowel was such as to distend it markedly. In certain animals which had taken water shortly before carbonate the ordinary dose of this latter caused no change whatever in the surface hue and there was either no change in the blood plasma relationship or practically none.

There exists a considerable literature describing blood acidosis as a complication of clinical anhydremia (12). In order to find whether anhydremia was the cause of the paradoxical tissue acidosis in our rats receiving carbonate, recourse was had to experiments in which the condition was produced by other means. Active purgation with N/1 magnesium sulfate soon proved effective, as disclosed by hematocrit tests, and there developed with it an acidosis of the superficial tissues. But a gradual respiratory failure took place at the same time and might have been largely responsible for the acidotic change. No such complication was observed with N/1 sodium sulfate. A rat of 138 gm. given 20 cc. of it in the course of 2½ hours, and purging actively as result, stained orange when injected with phenol red an hour later whereas it had stained red at a previous injection. The animal was now semiprostrate with exaggerated respirations. The hematocrit showed that 56 per cent of the blood volume consisted of cells, the normal proportion being between 40 and 45 per cent. The experiment was not repeated since a simpler method to produce anhydremia,—and outlying acidosis,—was found in the administration of hypertonic sugar solutions.

*Experiment IV. Anhydremia,—and Outlying Acidosis,—Following the Injection of Hypertonic Dextrose Solution.*—The general procedures were identical with those of Experiment III except that 5 to 10 cc. of a 50 in 100 solution in water of Merck's highest purity anhydrous dextrose was administered instead of carbonate; and the symptoms manifested by the rats were practically the same. The change from red toward yellow was swifter, though, and more considerable. The animals frequently died within an hour. Often before collapse occurred the rat was a brilliant orange-yellow, of such a hue indeed as was not attained by stained controls until a half-hour or more after they had been killed (by a blow on the head). Of those which survived some failed to alter in hue or became only slightly or transiently yellower. They purged a thin fluid stained yellow with phthalein.

The same changes in the hematocrit findings occurred as in the carbonate animals, and in the same general parallelism with the degree of tissue acidosis. But whereas in the carbonate instances the reaction of the blood either failed to alter or became more alkaline, in the sugar ones, though occasionally constant, it tended ordinarily to fall somewhat (in the most extreme instance from approximately pH 7.4 to pH 7.1). In rats that survived and manifested slight tissue acidosis or none, the pH of the blood did not change and the cell-plasma relationship was little affected.

In these instances of anhydremia caused by the ingestion of sugar the blood pH ordinarily fell. The change taking place in the superficial tissues might be deemed merely an expression of the blood alteration were it not far more considerable,—from about pH 7.4 to pH 6.4, to judge from the colors, as one is justified in doing (13). In papers already published we have shown that in normal animals changes produced in the reaction of the blood by the direct introduction of alkali or of acids, including $H_2CO_3$, are closely attended by changes of the same degree in the reaction of the subcutaneous tissue (8, 14). But the alteration occurring in this tissue when sugar has been given by mouth is out of all proportion to that in the blood, and hence it falls in the category of outlying acidosis.

*Averting Anhydremia Averts the Acidosis.*

In the course of the experiments with carbonate and sugar there had been numerous indications that when the dose of hypertonic material is insufficient to produce anhydremia, or when this last is averted through purgation and diuresis, outlying acidosis fails to occur. In amplifications of such findings some direct attempts were made to prevent the development of anhydremia. The results demonstrated that in proportion as they were effectual so too was acidosis lessened or prevented.

*Experiment V. Prevention of Outlying Acidosis by Water Injections.*—Ordinarily, for each test, four or six rats of identical weight were employed which had been urethanized and stained with phenol red. The same dose of N/1 sodium carbonate was given to all, and they were paired for intensity of color,—whenever any differences existed,—and laid on the back, side by side, upon the warm pad. A few minutes later the slow injection was begun of warm distilled water into the peritoneal cavity of one animal of each pair, through a hypodermic needle connected with a burette. The blood was followed, by the methods already described, with specimens taken from the carotid.

Clinical experience has shown that it is often difficult to check the course of anhydremia; and our efforts with animals given carbonate were only successful with critical doses of this latter. When more was given the desiccation and acidosis, which developed rapidly, were uninfluenced by water; when less, none of the animals became sufficiently anhydremic and acidotic for the purposes of the work. It would have been necessary under test-tube conditions to have added nearly 4 cc. of water to each cc. of N/1 carbonate in order to render the solution isotonic with the blood. To introduce the needed total into rats was manifestly impossible. But when the carbonate dose had been rightly chosen it was found that the gradual introduction of 10 to 18 cc. of water saved the animals from anhydremia, with attendant tissue acidosis, a condition to which controls succumbed. The fluid was rapidly removed from the peritoneal cavity of the injected rats, there was slight change at the most in the cell-plasma relationship, the blood became more alkaline than is normal, and alkaline purgation and diuresis took place, with rapid decolorization and recovery.

Similar but less extensive experiments in which dextrose was used instead of carbonate gave results that were similar except that the blood of the animals receiving the water did not become more alkaline than normal.

Better results in the prevention of anhydremia could have been obtained, doubtless, by the utilization of salt solution (12); but with the salt there would have been introduced a fresh difficulty in the interpretation of the experiment.

### *Outlying Acidosis after Hemorrhage.*

Anhydremic animals remain stained with phenol red long after the controls have decolorized. This indicates that there is a considerable interference with fluid interchange, as does the fact that the blood fails to become more alkaline when the dose of hypertonic sodium carbonate solution is very large. In the fulminant anhydremia, accompanied by tissue acidosis, resulting from such a dose the passage of fluid is all one way, so to speak,—into the gut. According to the literature the acidosis of the blood encountered in clinical anhydremia can be referred to metabolic disturbances arising from a diminished volume flow through the organs; and this in turn is due to a vasoconstriction compensatory for the lessened blood bulk. If the tissue acidosis is referable to diminished volume flow then it should develop when the blood bulk is diminished in other ways, as for example by hemorrhage. Such is the case.

*Experiment VI. Outlying Acidosis as the Result of Hemorrhage.*—The methods of staining, examination, etc., were identical with those in the preceding experiments. Rats anesthetized with urethane were bled from the carotid by the method employed to obtain blood samples.

It was soon found that large depletions were required to induce tissue acidosis. For instance in one rat out of a series weighing 136 gm. that were urethanized, fasted 18 hours, and bled repeatedly from the carotid at short intervals, the removal of 0.8 cc. of blood caused only a dubious change in the surface color. In a second animal bled 1.0 cc., the red hue altered definitely toward yellow; while two others losing respectively 1.4 and 1.6 cc. became notably orange, though the blood pH did not alter from its initial value. In the instances last mentioned the hematocrit findings at the time when tissue acidosis was well developed showed that the blood bulk had been but partially made up, the cell percentages having dropped only from 51 per cent to 49 per cent and from 57 per cent to 50 per cent respectively. The animal that lost most blood retained the orange-yellow hue and died some hours later.

Numerous other examples of the sort could be described. Decolorization failed to occur in the bled animals within the usual time, and in extreme instances of depletion involving tissue acidosis the blood pH often fell slightly. If the rats had taken water shortly before they were bled the loss in blood volume was made up more or less completely, and in proportion as this happened acidosis was transitory or failed to occur.

Urethanized animals stand bleeding but poorly and when acid is gradually introduced into the blood stream there is little or no compensatory blowing off of carbon dioxide (8). For these reasons the experiments were repeated in the absence of the anesthetic. Blood was removed by aspiration from the heart. The findings were essentially the same as after urethane.

Here again was a frank tissue acidosis unaccompanied by change in the blood reaction. In those extreme cases in which the blood pH fell, the fall in pH occurring in the tissues was several times more considerable, as disclosed by their color.

### *Characteristics of the Outlying Acidosis.*

The surface changes in hue of the phthalein-stained animal after hemorrhage, or during the development of carbonate anhydremia are of like character. When taking place gradually they exhibit a significant sequence. One notes that the body is becoming irregularly blotched, or mottled, as the result of local alterations from red toward yellow in the hue of the stained tissue. The blotches are ill-defined to begin with, asymmetrical, irregularly oval or rounded, and they vary

less in size than in color with the degree of the depletion. Those seen on the chest, abdomen, and thighs of shaved rats are from ½ to 1 cm. in greatest diameter, scattered upon a red background. They are usually discernible in smartly bled animals some 5 to 10 minutes after the depletion. When the blood bulk is poorly made up they grow progressively larger and more yellow. Meantime the regions between them become less red; and after a greater or less time, by enlargement and coalescence of the patches and by the just mentioned change in color of the interspaces, the orange-yellow hue becomes general. But before this happens the animal is often pronouncedly mottled in orange-yellow against red, a mottling which extends to the tissues still protected by hair as can be shown by stripping the pelt. Where the hair has been shaved off the patches are beautifully seen by transmitted light. Chilling of the skin is not the cause of them. For they occur in animals immersed in warm oil, and fail to develop in stained and urethanized controls placed in the ice box at 0.5°C. and succumbing in the course of an hour or more to the exposure.

If the depletion by hemorrhage has been extreme, or a fulminant anhydremia has been induced, a general acidosis of the surface tissue develops, with scant signs of patching. The time needed for the color changes implicit in the patching is enough in such instances for a diffuse alteration. Patching has never been encountered in rats rendered anhydremic with sugar; and the generalized change in hue occurring in such animals before death is, as already stated, far greater than under ordinary circumstances of diminished blood bulk, the color attained being nearer to yellow. There are, of course, special metabolic conditions to account for this. On the other hand a state of depletion can be induced with carbonate or by bleeding which leads to the development of mottling only, and this may persist for a long time without essential change in the situation or shape of the blotches. In such cases gentle massage will often suffice to bring back the red color to individual patches and obliterate them; but later they reappear. When they are merely a transient stage in the rapid progression to a generalized acidosis massage is without effect.

In animals that are recovering the patches grow smaller by a peripheral encroachment of red, and there goes on within them at the same time a reversion to this hue.

According to Kendall (15, 16) the adsorption *in vitro* of phenol red upon tissues is greatest when they are relatively acid; and we have noted this to be true of some of the other phthaleins as well (13). The factor seems to be without influence on the character of the phenomena witnessed in the living animal and just described. There is, furthermore, no evidence of a special destruction of the dye in regions that are poorly oxygenated. When the pelt is stripped back in depleted animals showing a patchy acidosis the rose-red to which the phthalein attains is as intense where the patches were as elsewhere. Incidentally it should be said that the change to alkalinity thus attested takes place also when the acidosis is generalized throughout the superficial tissue after bleeding or sodium carbonate; but that when sugar has been used as the depleting agent the orange hue is often slow to alter, the color not progressing beyond orange-red.

*An Outlying Acidosis Due to Intercurrent Pressure Differences.*

In extension of the observations a series of rabbits were depleted by hemorrhage. Elsewhere we have reported on the local acidosis developing in such animals when deeply under the influence of ether or urethane. No such acidosis is met in intact animals properly anesthetized with these agents, but the characteristic patchy mottling can readily be induced in them by bleeding. It was deemed best to avoid the influence of general anesthesia. For this reason a carotid was cannulated under ether some hours or a day beforehand and when the animal was fully recovered it was bled repeatedly, with the aid of novocaine locally. Under such circumstances, as the shaved, stained, and depleted rabbits sat quiet in the ordinary hunched position, a type of acidotic patching was seen to develop wholly distinct from that thus far considered, and preceding it slightly in point of time. This patching was localized to the surface regions overlying the bony prominences (knees, backbone), regions which had colored quite as rapidly as had those elsewhere immediately after the injection of the phenol red, and indeed sometimes in our experience slightly more rapidly. The change could readily be demonstrated by pulling to one side the loose skin from over the spine, for then there was brought to view a broad orange band which had lain over it. Rats are not so well suited to a demonstration of the sort but with them another

example similar in its essentials was repeatedly met. In urethanized individuals given a hypertonic solution and placed on the back the intestinal coils in which fluid accumulated out of the body soon raised the abdominal surface here and there, and over these slight protrusions the color of the stained surface tissues turned toward yellow.

Frequently in the crouching, depleted rabbit a slight, generalized change toward yellow, localized to the tissues of the lower part of the sides, preceded the characteristic patchy acidosis. The patching was never so pronouncedly orange-yellow as in rats.

*Relation of Local Ischemia to the Acidosis.*

An evident cause for the color changes over bony projections and low on the sides of rabbits is to be found in the existence of slight pressure differences,—differences devoid of effect under ordinary circumstances. We have already given reasons for the supposition that the ordinary patchy acidosis of depletion is referable to a lessened volume flow resulting from compensatory vasoconstriction. To learn how far the circulation was interfered with recourse was had to the injection of a second highly diffusible vital stain into animals in which a first one (phenol red) had already disclosed the acidosis.

*Experiment VII. The Patchy Outlying Acidosis Is Due to Patchy Ischemia.*— Rats and rabbits were stained and depleted in the ordinary way and then injected with a solution of purified brom phenol blue (Hynson, Westcott and Dunning). This indicator has a range too far to the acid side for its color to be influenced by the reaction of the tissues. We have found that a 4 per cent solution of it in water, brought to pH 7.4 with NaOH and rendered isotonic with the blood by the addition of salt solution,* is an excellent vital stain, producing no symptoms, and coloring the normal animal an even deep blue by diffusion into the tissues, within less than 5 minutes after its intravenous injection. 0.4 cc. suffices for the purpose in the case of a rat of 150 gm. and 3 cc. per kilo for rabbits. The staining of the tissues persists for many hours. In a succeeding paper the characters of the dye as a vital stain will be dealt with.

In normal animals colored with phenol red the introduction of brom phenol blue causes the hue of the body surface to change rapidly from red to a deep plum-purple; and the change takes place evenly. In animals with a patchy acidosis,

* 400 mg. of brom phenol blue is ground in an agate mortar with 40 mg. of sodium chloride and 1.2 cc. of N/1 sodium hydroxide. The mixture is brought to 10 cc. with distilled water and filtered.

however caused, the alteration is much more gradual and is highly irregular owing to the fact that the brom phenol blue fails entirely to enter the orange (acidotic) patches. These retain their hue whereas the red-stained tissue between them

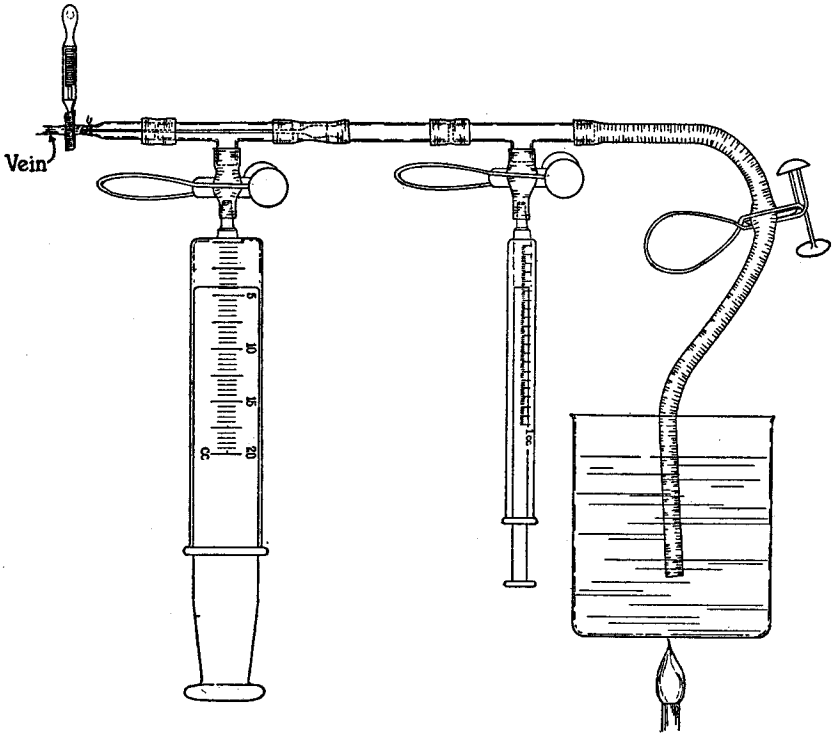


FIG. 1. Apparatus for the injection of a small quantity of warm solution into a tiny vein.

The injection apparatus is first brought to body temperature by drawing the warm dye solution through it by suction on the 20 cc. syringe. The solution passes from beaker to syringe by way of a capillary pipette extending inside the cannula nearly to the tip. When enough has been drawn through to warm the system the connections with beaker and 20 cc. syringe are rapidly clamped off and the injection is made by forcing the desired quantity of solution from the small syringe into the system, thus driving an equivalent amount of the warm fluid from this latter into the vein.

turns purple. Thus, within 10 minutes as a rule, the animal appears spotted all over with orange blotches on a plum-purple background, the blotches being separate or more or less confluent, according to the degree of the depletion. A later progression through the stage of generalized acidosis to that of death of the animal

is without the least effect on the particolored mottling. For with the increasing failure of the circulation the brom phenol blue remains stranded in the regions to which it had at first been distributed; and such secondary color changes as come about in the phenol red in response to the general acidosis do not suffice to alter the patterning. On the other hand in animals recovering from depletion the orange patches gradually merge with their surroundings and disappear as the blue dye still present in the blood enters them. In rats killed at the height of the patching its pattern is long maintained, being lost partially and little by little by diffusion of the blue dye.

On stripping the pelt it could be seen that the furred regions were affected equally with those that had been shaved. There was even a fine mottling of the ears and of the soles of the feet. In not a few cases phenol red was dispensed with. The depleted animals then appeared blotched with white on blue, and the control ones an even and deeper blue.

To test whether the superimposed brom phenol blue had any influence to determine the blotching sodium indigotate (the sodium salt of indigodisulfonic acid) was sometimes substituted for it. In order to color the animal deeply without introducing too much fluid a 2 per cent solution in water was used. The dye precipitates out at room temperature so a special cannula was devised to ensure that it was warm when it entered the femoral vein (Fig. 1). In depleted rats given no phenol red but injected with 1 cc. of indigotate the characteristic mottling, this time of white on indigo blue, was obtained. It proved possible to preserve the mottled pelts in acetone, after they had been rapidly removed and stretched.

### *The General Blood Pressure during the Local Ischemia.*

Here is proof that the patchy outlying acidosis is associated with a failure of fluid exchange within the patches, a failure so entire that highly diffusible dyes cannot enter them. The ischemia responsible for the patching would seem to be as complete as that induced by epinephrin and comparable in this respect to epinephrin ischemia. The question arises of the way in which it comes about. Can one suppose it due in part to inherent local differences in the conditions making for interchange between blood and tissue? No signs of any such differences are to be seen in normal animals during the process of vital staining. The surface tissues color evenly. But it might well be that under the circumstances of a greatly diminished blood pressure local influences of no moment ordinarily would become effectual. An example of such a phenomenon has already been described in the influence of prominences beneath the skin to determine regions of local acidosis in depleted rabbits. To decide the matter observations were

made on depleted animals to find whether the acidotic patchiness develops while the general blood pressure is still well maintained. This proved to be the case.

*Experiment VIII. The Blood Pressure in Animals with Outlying Acidosis.*— To determine the blood pressure the carotid artery was entered in the manner already described, with a needle of rather large bore (No. 23), mounted upon the barrel of a tuberculin syringe which in turn was connected by means of a water column with a manometer of about 1 mm. bore containing mercury (Fig. 2). Just before the needle was introduced some three-fourths saturated $MgSO_4$ was drawn up into it to prevent clotting and the mercury column was adjusted to

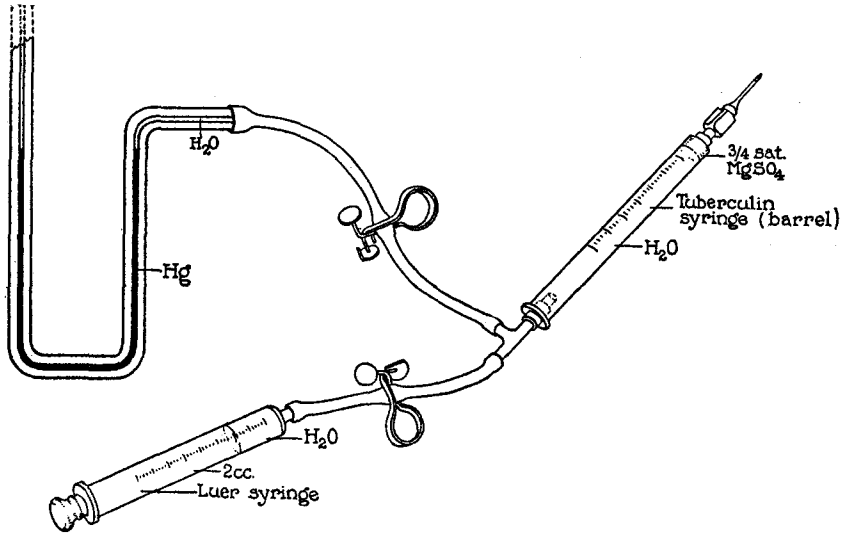


FIG. 2. Apparatus for determining the blood pressure of rats.

slightly less than the expected pressure. In this way the loss of blood was minimized. It could never have been more than a few hundredths of a cc.

The rats were urethanized, stained, and depleted by the administration of hypertonic sodium carbonate solution. A grave objection to the method was found in the progression of the anhydremic condition during the period in which local acidosis was developing to the point at which it became evident in a change of the surface color. Even when a local ischemia is induced with epinephrin the bloodless state endures for some minutes before sufficient acidosis develops to bring about a color change. It follows that under the circumstances of a progressive anhydremia the blood pressure prevailing when local acidosis becomes manifest must frequently be lower than at the moment when the local ischemia responsible for said acidosis comes into being.

The blood pressure in rats that were merely urethanized and stained ranged between 100 and 120, averaging about 105. In a fasting animal of 185 gm., given 3½ cc. of sodium carbonate, a characteristic patchy acidosis had developed after 20 minutes although the pressure was then 112 mm. Hg. 20 minutes later still the dose of carbonate was repeated, with result that after the same interval of time, or an hour in all, the spotting had become very pronounced. The pressure was now 67 mm. Hg. In a second rat of the same weight given only 3.5 cc. of carbonate a marked patchy acidosis was present after 20 minutes, and the blood pressure was found to be 91. In a third animal it was 88 after 25 minutes, when patchy acidosis had appeared. By the time a generalized acidosis had developed in these rats, and in others, some of which were depleted by bleeding, the pressure was lower, usually about 65 mm.

The carotid blood pressure of rats proved surprisingly high; and it was still well maintained after bleedings which sufficed to induce a patchy outlying acidosis. One is justified in the view that this last is referable to a patchy vasoconstriction, not to intrinsic local differences in the conditions determining interchange between blood and tissue.

The diffuse acidosis of the subcutaneous tissue observed as the result of large bleedings may be in part the result of a lessened oxidation traceable to the diminished number of red cells. But the relative unimportance of this factor is proved by the disappearance of the acidosis as the blood undergoes the dilution incident to restoration of its bulk.

*Distribution of the Ischemic Patching.*

Thus far we have confined ourselves to the happenings in the superficial tissues. But during the course of the work numerous evidences were obtained that an ischemia of the sort responsible for patchy acidosis of the subcutaneous regions occurs elsewhere. To study this ischemia proved easier than to study such acidosis as may have resulted from it. For the reaction of most of the organs is too far to the acid side for phenol red to be useful with them; and, as has already been stated, the color changes with brom cresol purple are frequently not clear-cut. One can readily tell, however, where local ischemia prevails by vital staining with brom phenol blue. That acidosis often results from the ischemia is a fair inference, even though it cannot always be disclosed by the indicators at present available.

We shall here confine ourselves to the phenomena occurring in animals depleted for the study of outlying acidosis. In later papers the distribution of the ischemia consequent on various disturbances will be dealt with at length.

*Experiment IX. The Distribution of Ischemia in Depleted Animals.*—Animals stained with phenol red were depleted by bleeding or by anhydremia induced with sodium carbonate. When a patchy acidosis of the superficial tissues had disclosed itself they were injected intravenously with 4 per cent brom phenol blue, and killed some minutes later by decapitation, the object being to remove as much as possible of the circulating dyes. The organs were inspected swiftly and the order in which they were looked at was varied from animal to animal.

At first urethanized rats and rabbits were used. After a considerable number of experiments had been performed the papers of Henning (17) and of Florey and Marvin (18) appeared, in which proof is brought that urethane interferes with some at least of the vascular responses. This rendered necessary a repetition of the work in the absence of a general anesthetic. Rats were depleted by aspiration from the heart and injected into a superficial leg vein. A special technic was devised in order to bleed the rabbits. Under ether anesthesia a common carotid was freed but not severed, and brought to the surface; a paraffined cannula was introduced into it, and the muscles were sewed together beneath in order that it should remain superficial. To keep it closed it was tied down with coarse silk upon a short piece of rubber tubing, the spring of the tubing against the soft ligature being sufficient to occlude the lumen. The incision was then closed loosely. When bleeding was to be done some hours later the tissues were moistened with novocaine as they were exposed, and the ligature was cut against the rubber. When the bleeding had been finished and a new ligature put on, the cannula was washed free of blood with salt solution. In this way the vessel could be opened and closed again and again, with the rabbit in a sitting position. It was found that the gradual removal within half an hour of somewhat less than half the total blood volume gave rise to a patchy change toward orange here and there on the body surface. The color differences were never so pronounced as in rats, and much more depletion was required to produce them. The rabbits exhibiting them still sat or moved about as normally. Warmed 4 per cent brom phenol blue was now injected into an ear vein and 3 minutes later the head was taken off at a blow. When experience had shown how much depletion was needed to induce local ischemia the staining with phenol red was frequently omitted.

The findings in the superficial tissues have already been described. As remarkable a state of affairs was encountered in the voluntary muscles. These color up evenly and rapidly with brom phenol blue under normal circumstances, and by the end of 3 minutes they are everywhere blue. But in bled or anhydremic rats, and in bled rabbits, staining takes place more slowly and according to a definite pattern, with many unstained regions regularly interspersed amongst the blue

ones. When phenol red has been omitted the long muscles appear transversely banded with blue. The bands are slightly zigzag, with the blue fading gradually at the edges. The mackerel sky appearance to which they give rise can be seen without dissection in the quadratus lumborum when the peritoneal cavity is opened. It is also visible in some muscles that are more or less flat, as in the pectorals and the gracilis. In sheet muscles such as the external and internal oblique there is, instead of the banding, a regular dappling of oval unstained areas on a blue background, or, when depletion has been great, a mere reticulation of blue on muscle tissue that is for the rest unstained. The amount of unstained tissue varies in other words with the degree of depletion. In rats injected with phenol red only, and then bled or rendered anhydremic the presence in the muscle of spots yellower than their surroundings, relatively acid that is to say, can with some difficulty be made out. A superimposed staining with brom phenol blue discloses the fact that this acidosis affects regions not entered by the blue dye. When depletion has been great the muscles stained with phenol red are orange-yellow throughout.

No study of the kidney was made. After many observations on the other large viscera we can say definitely that patchy ischemia fails to develop in them, even when the blood bulk has been so greatly diminished that death inevitably follows within a brief time. The spleen frequently appeared practically unstained with brom phenol blue, standing out vividly red against its dark colored surroundings. This evidence that the organ is shut off from the circulation in depleted animals is what might have been expected from the studies of Barcroft.

In the cartilage of the ears of depleted rabbits stained with phenol red patchy acidosis does not occur but there develops a generalized one, the tissue turning orange-yellow. It stains slowly but evenly with superimposed brom phenol blue. Acidosis was not to have been expected in the tendons since even mechanical interference with the circulation fails to cause it, for a long time at least; and none was found. Brom phenol blue failed to disclose any patchy ischemia of the bone in those few osseous regions into which the dye penetrates during the brief period after its injection.

These observations make plain that the contraction of the vascular bed which occurs in compensation for a diminished blood bulk affects the organs not only to different degrees,—a fact long known,—but in different ways. In the subcutaneous tissue and skeletal muscle of moderately depleted animals there occurs a well-ordered patchy ischemia so complete that the regions affected are to all intents and purposes no longer served by the blood. In consequence they become relatively acid. Lying between the acidotic areas are others in the same tissue which manifest the normal reaction and are by comparison excellently supplied with blood. The viscera show no patchy ischemia visible in the gross, but the entire spleen is often deprived of circulation for long periods.

## DISCUSSION.

The direct proof that acidosis develops during life in tissues ill served by the blood merely substantiates a conclusion reached in the past on excellent indirect evidence. But such evidence has not enabled investigators to tell when local acidotic states will develop, much less anything about their distribution, intensity, and course. The method of vital staining with indicator dyes yields information on all these points.

Previous experiments (8, 14) have brought out the fact that changes induced in the reaction of the blood by the direct injection into it of acid or alkali, by breathing high percentages of carbon dioxide, or by hyperventilation are all closely accompanied by changes of essentially the same magnitude in the reaction of the subcutaneous tissue. In the present paper the existence is described of conditions in which a parallelism of this sort fails to obtain. Often a frank and not inconsiderable tissue acidosis develops, unassociated with any alteration in the blood reaction or with a slight one at most. It can even happen that the blood becomes more alkaline while the tissues are growing acid. Though the determining causes for these discordances are various, those that we have studied have a common physiological basis in circulatory shortcomings.

The observations on epinephrin ischemia have shown that where the circulation to the subcutaneous tissue of rats is interrupted a relative acidity develops within but a few minutes. When the blood volume of an otherwise normal animal is abruptly diminished by hemorrhage or by the administration of a hypertonic solution there develops a widely distributed tissue acidosis even though the general blood pressure is still well maintained and the blood pH unchanged. Such acidosis is traceable to the same cause as that due to epinephrin, namely vasoconstriction. This is not to say that the method whereby vasoconstriction is induced is necessarily devoid of any influence of its own. Animals depleted with hypertonic sugar solution show a more pronounced tissue acidosis than those given hypertonic sodium carbonate or bled; the acidosis tends to be more widely distributed throughout the subcutaneous tissue; it is more often accompanied by a lowering of the blood pH; and the acidotic tissue when exposed to

the air reverts toward alkalinity slowly and slightly. There exist numerous reports on the complex metabolic disturbances caused by introduction into the body of sodium carbonate or bicarbonate. The lactic acid of the blood is increased in quantity (19–21), and acetone bodies are voided in the urine (22). From the protocols of some of the work one can perceive that so much carbonate was given as must inevitably have led to anhydremia, local ischemia, anoxemia, and acidosis. For nearly 40 years it has been known that conditions of anoxemia result in a formation of lactic acid (23). But in the most recently described instances of carbonate ketosis, anhydremia can scarcely have played a rôle.

One would expect that in anhydremic animals the increased viscosity of the blood would act as an adjuvant to vasoconstriction and local ischemia would occur under circumstances of less reduction in blood volume than when hemorrhage is the effectual agent. We have gained the impression that this is so, without having proved it.

Outlying acidosis must develop in numerous pathological conditions of man which are attended by faulty interchange between the blood and tissues. The literature gives one every reason to suppose it a not infrequent complication of the clinical anhydremias. What is known of the physiology of shock, when viewed in the light of our findings, justifies the belief that the blood acidosis encountered in that state but feebly expresses the degree of acidosis existing in certain of the tissues. The same is true of the blood changes observed after severe hemorrhages. Hertzmann and Gesell (24) have noted that on suddenly restoring the blood volume after bleeding a temporary fall in the pH of the fluid may occur. The suggestion has been made (25, 26) that local acidotic accumulations may suddenly be swept into the blood with result in harm to the organism. Our observation, that the ventilation of tissues which have become acidotic as the result of simple ischemia brings about a rapid reversion in them toward alkalinity, would seem to indicate that the relatively innocuous carbon dioxide plays a major rôle in determining local acidity under such circumstances. In the failure of this reversion to occur in animals rendered anhydremic with sugar one may perceive evidence of the formation of non-volatile acid substances.

The question whether long continued local acidosis brings about

local pathological changes is not without clinical implications. In this connection some observations of the present work may be recalled, in which regions of local acidosis over knees and spine were determined in depleted animals by pressure differences of no consequence under normal circumstances. It seems possible that similar pressure differences, coming into play under pathological circumstances, might explain the localization of certain skin lesions seen clinically. But while the pressure factor may well be of importance here, local acidosis as such will scarcely suffice as a cause for the lesions themselves. The sedentary clerk sitting all day upon tissue that is inevitably ischemic and acidotic sustains no local injury from this cause. Connective tissue cells continue to live and proliferate after they have rendered the medium about them markedly acid (27), and they will withstand much larger alterations in reaction, produced with $CO_2$, than any attained in acidotic tissues (28, 29).

Many cells throughout the body normally contain vacuoles that are acid in reaction; and both polymorphonuclear and mononuclear phagocytes elaborate an acid material around particles that they engulf. Indicators of suitable range when taken up by such cells show the hue of acidity so long as these elements live, but that of alkalinity when, dying, they become permeable to the alkaline fluids round about (30). Such observations cause one to ask whether local tissue acidosis may not develop in the absence of circulatory derangements. The evidence we have gained by altering the blood pH through the introduction of acid or alkali, showing, as this did, rapid and precisely concomitant changes in the reaction of the matrix tissues, is all against any such happening in the intercellular substances. On the other hand enormous accumulations of relatively acid material may occur within cells, as is evident when they segregate litmus (30), the peritoneal coat, for example, then being much thickened and colored deep red by elements which have converted the indicator from the alkaline form and stored it. A segregated acidosis of sorts here surely exists as it must under other circumstances. Of the protoplasm itself nothing is known in this relation. The color of living parenchymal cells (liver, pancreas) stained with a phthalein indicator does not alter when enough acid or alkali is introduced into the organism to bring about pronounced changes in blood pH and the pH of the matrix tissues.

One would expect the speed with which outlying acidosis develops, and perhaps its intensity, to be less in rabbits than in rats, since the smaller the mammal the higher ordinarily is its metabolic rate. And this is the case. Rabbits stained with phenol red and depleted change color slowly as compared with rats, and the color attained is never so yellow. In the stained dog an ischemia produced with the tourniquet or with epinephrin results in an evident tissue acidosis only after the lapse of 15 minutes or more. In two dogs repeatedly bled under ether no patchy acidosis could be elicited, but a slight general one became evident after a considerable interval. On the other hand patchy ischemia with acidosis is readily induced in the cat by bleeding, as one of us, with Dr. H. P. Gilding, has recently found. The time required for the development of the acidosis seems to be much the same as in the rabbit. A young white pig which had shown pronouncedly the reactive hyperemia of Bier (31), and the stroking phenomena intensively studied by Lewis (32) was injected with phenol red and bled under ether in the attempt to bring out a superficial patching. None occurred, nor did the skin as a whole change color though the depletion was pushed so far that the animal died. Not until nearly an hour after death was a slight change in the body hue noted, such as would accompany a fall of pH 0.1. It follows that a patchy ischemia may have been present during life without causing sufficient local acidosis for it to have become evident through a color change. The animal was very fat.

Clinical reports on cutaneous phenomena, when viewed in connection with our findings, give one every reason to suppose that a local ischemia with acidosis occurs not infrequently in man. Unfortunately no indicator is as yet available for utilization in this connection. The pale orange hue assumed by weak solutions of phenol red injected directly into superficial acidotic areas is too frequently masked by the skin color to be generally helpful.

The patchy ischemia disclosed by staining methods in depleted animals is one expression of a widespread and well-ordered circulatory readjustment obtaining under emergency conditions. It seems not to have been recognized before. A further report upon it will be published shortly.

SUMMARY.

In various functional conditions involving peripheral vasoconstriction a more or less widespread change toward acidity takes place within certain tissues. The change is frequently independent of any in the blood. Indeed the blood can become more alkaline while the tissue acidosis is developing.

When the blood volume is diminished abruptly but not too greatly, by hemorrhage or by anhydremia, the acidosis which develops in the superficial connective tissue and in the skeletal muscles is patchy in distribution, being limited to areas of local ischemia themselves the result of a compensatory vasoconstriction which affects certain regions only. There is a second type of patchy ischemia (and of acidosis) which occurs under circumstances of moderate depletion and is referable to local pressure differences that are so slight as to be ineffective under normal circumstances. A generalized acidosis throughout the superficial tissue develops when depletion is extreme. All these are outlying acidoses, since they lie without the influence of the blood. In the viscera no such acidoses have been found.

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