

of the same genus were examined and found to show very similar conditions. The genus has a very extensive distribution and may be obtained practically throughout the United States, being "very common in summer and early autumn in damp meadows and along the margins of streams and ponds."

Keys for determining material may be found in Blatchley's "Orthoptera of Northeastern America" and in Rehn and Hebard's "Synopsis of the Species of the Genus *Orchelimum*," Transactions American Entomological Society XLI.

In order to obtain many fine spermatogonial divisions, immature males should be killed with a few drops of xylol, the testes dissected out and fixed for three hours or longer in the following fixative, which must be freshly made up:

75 cc saturated aqueous solution picric acid, 15 cc formalin, 10 cc glacial acetic acid, 0.5 gram urea and 1.0 gram chromic acid crystals.

Wash in 70 per cent. alcohol until no longer yellow. Dehydrate clear, infiltrate with paraffin and section 7-8 μ thick. Stain in iron haematoxylin.

Ovaries should be dissected from adult females and treated as above. Since the larger eggs usually crumble when sectioned they should be removed before dehydration. Slides should be differentiated to show polar views of metaphases of dividing follicle cells.

The material is also very suitable for the demonstration of the distinctive differential behavior of the accessory chromosome in spermatogenesis. Since this element is so extraordinarily large its peculiarities in the prophase are particularly striking.

ROBERT L. KING

ZOOLOGICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA

SPECIAL ARTICLES

THE RELATIVE REACTION OF LIVING MAMMALIAN TISSUES

THE methods which have led to a comprehension of acid-base conditions in the blood are for the most part inapplicable to the study of the reaction of individual tissues in the living mammal. This field of inquiry has remained, not closed indeed, but well nigh unentered. The observations which follow indicate that the tissues in general are less alkaline than the blood, and that in some of the organs, on occasion at least, a notable acidity prevails.

Rats and mice can be vitally stained with litmus by repeated injection of it in the purified blue state. The coloration persists for weeks without manifest detriment to health. At first the whole creature be-

comes blue owing to the amount of indicator present in the body fluids; but soon a striking color differentiation develops. The hairless surfaces now appear violet because of a red-stained connective tissue overlain by a blue-stained epithelium. Long after the indicator has practically disappeared from the body fluids the surface epithelium, the bones, osteoid tissue, cartilage, tendons, aorta and heart valves are still diffusely blue, whereas other tissues, notably the connective tissue everywhere, the liver, pancreas and kidney are of a pronounced rosy red. The red color is almost wholly the consequence of a segregation of litmus in intracellular granules acid in character; and the amount of granular matter may become so great that the predominant reaction in the tissue when crushed is likewise acid to the indicator. In uninjured connective tissue a blue ground-work can be made out between cells laden with the red granules, whereas the parenchyma of certain of the viscera containing macrophages filled with such granules has a ruddy sheen, difficult to discriminate in the presence of these latter, which might be taken to indicate an acid state.¹ Cells with red granules dying here and there in the body are for a brief period colored diffusely blue; and local derangements within elements yet alive are signaled by a change in the tint of individual granules. The acidity of some at least of these granules in which litmus is stored is so considerable that when brom cresol green instead is deposited in them they are rendered yellow, a hue which, under controlled circumstances in the test tube, would indicate an acidity at least as great as pH 4.0.

The sodium salts of the phenol indicators stain living tissues far more rapidly, deeply and diffusely than does litmus; and several are well tolerated, as the event has shown. Mice given thymol blue, which is yellow at pH 8.0 and blue at pH 9.4, become yellow practically throughout, a fact which sufficiently indicates that the range of the indicator is too far to the alkaline side for it to be useful in the present connection. After cresol red (yellow at pH 7.2; purplish red at pH 8.4), the hairless body surfaces are reddish yellow, whereas the tissues exposed when the anesthetized animal is laid open have a clear yellow hue. The plasma of blood removed from the right ventricle into paraffined containers and under

¹ In 1913 (*J. Exp. Med.*, xviii, 183) the writer recorded the fact that some tissues grow well in plasma rendered acid to litmus through their metabolic activities; and Lewis and Felton, Fischer and Mendeléeff have since demonstrated that some survive at a pH as low as 5.5. Stieglitz (*Arch. Int. Med.*, 1924, xxxiii, 483) reports that the kidney cortex of dogs receiving azolitmin intravenously becomes red with the indicator in the course of a few minutes.

paraffin oil is by contrast yellowish red. This finding, of a less alkaline state in the tissues than in the blood, is confirmed with phenol red (yellow at pH 6.6; red at pH 7.8). The circulating fluids of mice injected with this substance are damson colored, whereas an intense yellow suffuses the tissues. With both indicators a significant phenomenon is to be witnessed during the dissection. Almost immediately on exposure to air the connective tissue stained with cresol red becomes more alkaline as shown by a change from yellow through red to rose-purple, while with phenol red not only this tissue but the cartilage, tendons and bony surfaces undergo alterations in hue that are similar in significance. Krogh² has shown the ease with which carbon dioxide passes through animal membranes.

Brom thymol blue, the next indicator of the series (yellow at pH 6.0; blue at pH 7.6), stains but poorly and produces an acid intoxication in mice, as is clearly attested by the yellow hue of both blood and tissues in the prostrated animals.

Brom cresol purple, which is yellow at pH 5.2, and purple at pH 6.6, stains the tissues intensely, but the dose necessary for the purpose comes close to that which is lethal. Through its use differences in the reaction of individual organs can be made out such as were foreshadowed by the work with litmus. In animals receiving it, the circulating fluids, the skin epithelium, connective tissue, bone, cartilage, tendons, aorta and heart valves are all rendered intensely purple, whereas the fatty tissue, striped muscle, liver, kidneys, lymphnodes, pancreas and certain other viscera exhibit various shades of greenish-yellow, changing to purple when alkali is applied. There can be no doubt that under the circumstances of the work a considerable acidity prevails in the last-mentioned group of organs. The color nuances to be observed in many regions of the body constitute a clear-cut tissue differentiation on the basis of differences in reaction. Pre-existing pathological changes are often sharply demarcated by colors that attest to abnormalities in the tissue reaction.

Methyl red is too susceptible to reduction for use during life; and brom cresol green, which possesses much the same range (yellow at pH 4.0; blue-green at 5.6), colors the tissues an intense blue-green, sufficient evidence that this range lies too far to the acid side for successful utilization.

The phenol indicators are excreted in large part through the bile; and the hue of the intestinal contents, which are stained in consequence, alters with the conditions at the different levels of the gut.

The information given by the indicators available

at present for injection into living animals must be accepted with caution. Even the best of them is influenced by the substances with which it comes in contact; and little is known of the effects on the body of the majority of them. But the findings here set forth will serve perhaps to point the way to more conclusive studies. They emphasize the need for color methods whereby the manifold physiological differentiations within the living body can be disclosed with something of the certainty that now obtains for morphological ones.

Details of the work will appear in *The Journal of Experimental Medicine*.

PEYTON ROUS

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH,
NEW YORK

DIRECT SYNTHESIS OF HIGHER FROM LOWER HYDROCARBONS¹

IN examining the stability of gaseous ethane at normal pressure under the α -radiation of radon (radium emanation) mixed with it in a small glass sphere (1.7 cm diameter), a liquid phase appeared after the first day's radiation. Since nothing was initially present in the glass vessel except ethane confined over mercury (having contact with the ethane in a capillary connecting tube) and the infinitesimal quantity of radon (222.5 millicuries = 0.12 cubic millimeters and a pressure of 0.8 mm of foreign gas), it was evident that ethane was being converted into one or more of the higher hydrocarbons. The quantity of liquid hitherto obtained is too small for positive identification, but has about the vapor pressure of octane, which is the lowest member of the paraffin series that would form a liquid having the low vapor pressure observed at room temperature.

If the product is octane, the reaction which apparently takes place is $4C_2H_6 = C_8H_{18} + 3H_2$, which might take place in two stages with butane as the intermediate product. It is also not impossible that the product may be an unsaturated or a ring compound produced by splitting out more hydrogen.

The liquid was reddish-brown in color, most probably due to colloidal carbon, which shows that decomposition into carbon and hydrogen must also take place to some extent. Abundant hydrogen was shown to be present. After a few days, during which time the total pressure continued to increase, nothing remained except hydrogen and liquid generally distributed over the walls of the vessel in fine droplets, and forming a minute liquid column in the capillary tube at the bottom of the vessel. The pressure con-

² Krogh, A., *J. Physiol.*, 1919, lii, 391.

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