

In recent years the only specific suggestion for staining old blood films seems to be the method of Daniels (1907) which, as outlined by Craig (1), calls for a preliminary acid-alcohol bath followed by washing and staining in the usual manner. Repeated trial of these methods did not give the desired result. While the gross appearance of the stained smear is more red with an acid buffer in the range mentioned and darker blue with an alkaline buffer, the red was chiefly that of eosin and the blue largely the result of precipitated stain. Our problem was particularly one of failure to obtain (red) staining of the chromatin. Microscopically, despite the red cast with acid buffers, it was the blue which seemed to stain the nuclei of the leucocytes, rather than the azure components of the stain.

This recalled that hematoxylin, also a nuclear stain, is retarded by acid and precipitated by alkali. Moreover, its staining action is hastened by preliminary rinse of tissue sections in the alkaline bluing bath. Trial of Wright's stain showed clear blue in acid solution but purple with a slight precipitate in alkaline solution. Methyl green was blue in acid but purple in alkaline medium. Azure II showed little change. Azure I and Azure B showed darker blue in the acid solution.

On this basis it seemed that azure-staining of the chromatin might be improved and undue precipitation avoided by using alkali as a preliminary bath rather than as a medium for the stain.

Actual use has verified this principle in several recent clinical cases where typical plasmodia could not be found by routine stain but were present, numerous and well stained after the procedure described.

Sodium carbonate and ammonium hydroxide have both been satisfactory. The concentration is best adjusted by pH determination. Fresh smears will not tolerate strong alkali, and brief exposure to pH 8-9 after fixation may suffice. Older smears are not damaged by considerably stronger alkali and seem to require it for comparable effect. Because of this variation it is not yet possible to be specific as to concentration or time. At present the strength is adjusted to give partial, but not complete, hemolysis. The slide is washed and then stained. For some, the usual time suffices, while for other bloods, prolonged staining is a necessary and helpful factor. This alone gives better results than the routine method, but the use of both the alkali and prolonged staining is distinctly preferable. The nuclei of leucocytes should be distinctly overstained, dark reddish-purple. The staining time is perhaps best controlled by this.

By using several hours in the alkaline bath and 24 hours in Giemsa stain, or a comparable solution of Wright's stain, the parasites brought from Texas in March 1944 have finally been stained to present the desired classical picture.

This seems too useful a tool to withhold, although it needs further development. The possibility of materially hastening the result by adding some penetrant (such as Tergitol-7) is being investigated.

References

1. CRAIG, C. F. *Laboratory diagnosis of protozoan diseases*. Philadelphia: Lea and Febiger, 1942.
2. MACKIE, THOMAS T., HUNTER, GEORGE W., III, and WORTH, C. BROOKE. *Manual of tropical medicine*. Philadelphia: W. B. Saunders, 1945.
3. RATCLIFFE, A. W. *J. Ind. St. med. Ass.*, in press.

Letters to the Editor

The Fluorescence of Radium Burns

The fluorescence of scar tissue has been scantily mentioned in past writings in this field. However, it is now widely assumed that cicatrices due to mechanical, electrical, and chemical trauma show little by way of a distinctive response to filtered, long-wave-length, ultraviolet light. Usually tissue damaged by these agents appears lavender or purple to the unaided eye. This excludes the possibility that such scar tissue may contain traces of porphyrins or related substances which would produce a bright red fluorescence, a subject yet open to investigation.

Moreover, it has been assumed that cicatrices due to the effects of X-ray and radium radiations are characterized by a brilliant pearly-white fluorescence under ultraviolet light.

The writer has personally observed numerous instances in which X-ray damage to tissue on various parts of the body fluoresced the pearly-white color. However, it has recently been possible for him to compare the fluorescences of X-ray cicatrices with those caused by radium. In two cases, both adult male and female, tissue damaged in radium therapy not less than 10 years previously exhibited a brilliant light-blue fluorescence under filtered, long-wave-length, *i.e.* 3,650 Å., ultraviolet light. In the case of the female, radium irradiation had been employed 15 years previously to remove a small growth on the cheek. The cicatrix was circular, showing a bright-blue fluorescent periphery and a nonresponsive center. The female subject was 25 years of age. In a 55-year-old male, radium had been used 12 years prior for treatment of a growth covering the lower, external

part of the nose. The damaged area also fluoresced the same blue, though the intensity was less pronounced.

From these observations it appears necessary to revise the existing concept of the fluorescence of scar tissue as it pertains to the etiologic factor.

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Interesting Observations in Dissections of the Frog

Medical men occasionally amaze the readers of newspapers and journals with reports of interesting anomalies of human anatomy. Students of general zoology are no less amazed to find that Mother Nature sometimes plays havoc with the internal anatomy of some of her lesser creatures.

In 18 years of directing laboratory dissections of the frog, numerous anatomical anomalies have been called to my attention by puzzled students. One of the most interesting cases was the Anuran specimen upon whom the students bestowed the curious name, "Glass-sides." Apparently, in the course of embryological development, the tissues normally laid down to form the laminated muscles, the obliquus externus and internus, had failed to develop. As a result, the internal organs were clearly visible through a thin sheet of mesentery-like tissue, occupying the normal position of the muscle, extending from the dorsal fascia to the linea alba. The animal had developed to normal size at maturity, and although the intestines were held in by little more than the skin, no ruptures had occurred.

Anomalies of the genital system are not common, but in one male specimen the right testis was lacking. In this same animal the position of the stomach was reversed from left to right.

In the above-mentioned anomalies and in numerous other cases of missing or misplaced organs the animals had developed to normal size and were not apparently affected by these defects.

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The Plainview, Texas, Fossil Bison Quarry

A fossil bison quarry at Plainview, in Hale County, Texas, discovered in 1944, was excavated during 1945 by the Bureau of Economic Geology of the University of Texas and the Texas Memorial Museum. The quarry yielded skeletons, in varying degrees of completeness, of between 50 and 100 bison of an extinct species appreciably larger than the modern buffalo. The bison skeletons were found in the filled valley of a stream at a depth of 12 feet. The unusual accumulation of skeletons may have resulted from a bison stampede. With the bison were found 19 projectile points and 8 other artifacts, chiefly or entirely scrapers. The projectile points, while resembling the known Folsom and Yuma points used by prehistoric hunters, are distinctive and have been named Plainview points (*Geol. Soc. Amer. Bull.*, 56, 1196).

Bison material to the amount of about three tons, as

packed for shipment, was removed from the quarry. Included were eight blocks of bones showing the full thickness of the bone bed. Two of these blocks, containing artifacts in place among the bones, will be placed on exhibit in the Texas Memorial Museum at Austin. With this new material the Memorial Museum will have exhibits of "Early Man" and associated fossils from four Texas localities: Malakoff, Henderson County; Cowan Ranch, Roberts County; Berclair terrace of Blanco Creek, Bee County; and Plainview, Hale County.

The only vertebrate fossil found immediately with the bison bones at Plainview is a large wolf, although the same deposits, near by, here yielded the Columbian elephant, *Parelephas columbi*, and a fossil horse, *Equus* sp. as well as an additional artifact, a scraper. Only fragmentary remains have been recovered of the wolf, and the species has not been determined. It is apparently smaller than the great wolf, *Aenocyon ayersi*, found with human materials at the Bee County locality and at Vero Beach, Florida (*Science*, 1916, 44, 615). The Columbian elephant has been found near, or in association with, human relics at all of the localities here mentioned.

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On "The Rumbling of Thunder"

In a recent article by Samuel R. Cook (*Science*, 1946, 103, 26-27) a "new cause" for the rumbling of thunder is described. He states that he believes this "new cause" to be "more potent" than any of the four causes usually named. This letter is not to dispute either the existence or potency of the new cause, but to raise some further possible questions concerning it.

In the first place, if we assume Mr. Cook's cause to be the only cause of rumbling, we would logically expect an even distribution of rumblings with the loud clap and diminuendo and those with a crescendo followed by a loud clap. We should also expect many in-between rumblings. This writer has not observed these phenomena in this locality.

In the second place, assuming the arrangement of electrons in the discharge as described ($N, 2N, 4N, 8N, \dots$), we wonder about the distances between the points where these charges occur. Let us fix the number of electrons at the second cloud as $2^k N$. Then, from the loudness of the clap and the rapid diminuendo, must we assume that the distance between the points of $2^k N$ electrons and $2^{(k-1)} N$ electrons is much shorter than that between the points for N and $2N$ electrons? Or are we to assume that the distances are random and that this can account for the fluctuations in the loudness of the rumbling?

Finally, the time of continuation of the rumbling needs some consideration. Let us assume two cumulus clouds 6,000 feet up and two miles apart, with the second cloud immediately over the observer—an extreme condition. Then the clap should be heard 5 to 6 seconds after the flash is seen, and the rumbling, if all is audible, for 11 to 12 seconds. But considering the