double impregnation silver method(1). In this mosaic it has been possible to demonstrate 16 boutons, while a single film would have shown 4 at most. All the processes of the Purkinje cells existing in the 15 μ section could have been followed out by this method if it had been required.

Summary. One solution to the problem of the photomicrographic demonstration of *Boutons terminaux* in the normal human brain is proposed in the form of in-focus mosaic photography.

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The Tetracaine Flare: Differences in Actions of Procaine and Tetracaine on Peripheral Nerve.*† (20508)

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It is well known that trauma of the skin or the intradermal injection of histamine results in the development of a flare, an area of erythema extending from the site of injection for several centimeters. The studies of Lewis(1,2) and others have established that the flare is brought about by a local nervous mechanism, the so-called axon reflex. In support of this concept is the observation which we have repeatedly confirmed(3,4) that previous infiltration of the skin with the local anesthetic procaine, in the area where histamine is to be injected, prevents the development of the flare, although the local wheal produced by histamine is not affected.

Our attention was drawn(5) to the paradoxical observation that tetracaine, a potent local anesthetic related chemically to procaine, itself produces a flare when injected intradermally even while causing gross anesthesia. This phenomenon appeared to offer the opportunity for testing the concept of the axon reflex as the basis of the flare and for further elucidating its mechanism. The present communication deals with this problem and offers the results of our attempts at its solution.

Material and methods. In all cases, injections of the various agents were made intradermally with 26 or 27-gauge needles in measured volumes from tuberculin syringes. Sterile crystals of procaine hydrochloride‡ and tetracaine hydrochloride‡ were dissolved in sterile isotonic saline to the desired concentrations, expressed as weight/volume. When either agent was injected alone, the volume was 0.1 ml. When the blocking action of procaine on the flare of tetracaine was tested, the former was first infiltrated into the skin in a volume of 0.2 ml, and a few seconds later 0.05 ml of tetracaine solution was injected into the center of the anesthetized area. In all experiments a control was obtained for the dilution factor by injecting the same volume of tetracaine into 0.2 ml of isotonic saline.

To determine the effects of procaine and tetracaine on pain thresholds, the drugs were injected in volumes of 0.1 ml into the skin of the volar surface of the wrist. The thresholds for "fast" (immediate) and "slow" (delayed) pain were determined by use of the warm-wire algesimeter of Lee, Williams and Pfeiffer(6). A bent resistance wire, heated by a battery to various temperatures indirectly indicated by an ammeter, was lightly and briefly applied to the skin. At sub-threshold temperatures only touch was felt. When the threshold for slow pain was reached, the pain was felt after an interval of about one second following the touch. Further increase in temperature finally resulted in elicitation of fast pain occurring simultaneously with the

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‡ Procaine (Novocaine) and tetracaine (Pontocaine) were generously supplied by Mr. Harold Epley, Winthrop-Stearns, Inc.

touch. When fast pain occurred it was usually no longer possible to discriminate the slow pain. Since the threshold for fast pain was always 30 to 50% higher than that for slow pain, however, it was assumed that whenever fast pain occurred the stimulus was above threshold for slow pain. When control determinations were being made the wire was touched to 5 different spots for each temperature. The threshold for each type of pain was taken as that ammeter reading at which the wire consistently produced pain in 2 or 3 of the 5 trials. After the anesthetics were injected, the thresholds were determined every 15 to 30 seconds until they returned to the control level. Since the thresholds changed rapidly, each test at a given temperature consisted of only two applications of the wire rather than 5, and if one of the 2 resulted in a positive response, that stimulus was considered to be at or above thresholds at that moment. In all cases, the thresholds for both types of pain were repeatedly checked during the experiment. The reported times for full recovery are accurate only to within 1 to 3 minutes. The concentrations were selected so as to give an effect lasting about 10 to 20 minutes.

Statistical significance of the results of the last experiment was determined in two ways. First, the mean differences between times for recovery of slow pain and fast pain (S-F) under procaine was compared with the mean difference under tetracaine by use of Fisher's t-test(7a). Secondly, the adjusted chi-square for a 4-fold table(7b) was determined where the two classes of the one attribute were *a* duration of effect on slow pain greater than on fast pain ($S > F$), and *b* ($S \leq F$); and the two classes of the second attribute were *a* procaine and *b* tetracaine.

Results. A. *The flare and its blocking by procaine.* Thirty-three tests were made with tetracaine at concentrations from 1% to 0.0001%. The threshold for production of flare appeared to be about 0.001%, since at this level 2 of 6 tests gave a positive response, while none of 4 at 0.0001% did so. At 0.01%, 4 out of 5 were positive; higher concentrations uniformly caused the flare. In other respects, excepting the local anesthesia, the response to tetracaine was identical with that of histamine: the local wheal, the surrounding hyperalgesia and "itchy skin" were all observed. Injections of 1% tetracaine caused definite irritation of the skin and in two cases a local necrosis and slough ensued. The flare produced by 0.1% tetracaine was roughly of the same magnitude as that of 0.001% histamine. Redness appeared within seconds of the injection, gradually increasing in extent and intensity to cover a roughly circular area of 2 to 5 cm diameter.

Ten tests were made to determine the effect of procaine on the tetracaine flare. In 2 cases, 1% procaine was shown to block completely the effect of 1% tetracaine; in 7 of 8 experiments 0.2% procaine blocked 0.2% tetracaine. No further attempt was made to establish dosage relationships between the two agents. Procaine itself produced only a local reddening at the site of injection, presumably due to its anesthetization of local vasoconstrictor fibers. B. *Effects of tetracaine and procaine on conduction in isolated nerve. Preliminary experiments.* Thus, it appeared that tetracaine, while producing a marked local anesthesia, can evoke a neurally mediated flare, and that procaine is able to block the flare. These facts suggested 1) that the fibers involved in the axon reflex for flare may be different from those which participate in the gross sensory response; and 2) that tetracaine and procaine act differently on the two groups of fibers, the former failing to block completely the one group, thus allowing the flare to develop, the latter effectively blocking all the fibers concerned. To test the latter suggestion, the effects of the two agents on conduction in the isolated, desheathed bull frog sciatic nerve were investigated.§ The results may be summarized as follows: 1. Tetracaine had an overall potency about 100 times that of procaine on a molar basis. 2. On washing away the anesthetic from a nerve whose conduction had been completely blocked, in the case of procaine (as is reported for cocaine (8)) the A fibers (rapidly conducting) recov-

§ These experiments were kindly performed by Dr. F. S. Crescitelli. Future definitive experiments are planned.

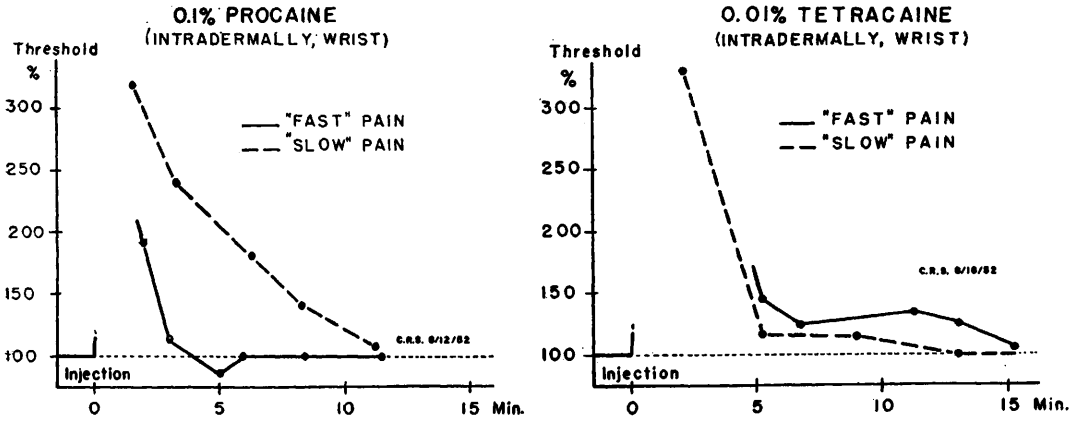

FIG. 1. a (left). Responses of slow and fast pain thresholds in a subject after injection of procaine. b (right). Ditto, same subject after tetracaine.

ered much sooner than did the C fibers (slowly conducting). The converse occurred with tetracaine in that recovery of the C fibers was more rapid than that of the A fibers. In the initial phase of blocking after application of the anesthetic to the nerve, corresponding effects were observed but the differences were not as marked: with procaine, the C fibers were blocked more rapidly than the A fibers; with tetracaine the opposite occurred.

These observations lent support to the suggestion that the two agents have different effects on conduction among the different groups of fibers, tetracaine manifesting much less blocking action on C fibers with respect to A fibers than is the case with procaine.

C. *Effects of tetracaine and procaine on thresholds of "fast" and "slow" pain.* The preliminary observations on isolated nerve, showing differences in action between the 2 local anesthetics, suggested a corollary experiment in the human skin. The "double response" of pain(9) presented itself as an ideal situation for testing function of A and C fibers. It has been inferred(9) that "fast" or immediate pain is mediated over rapidly conducting fibers probably of the A group, while "slow" or delayed pain is mediated over slowly conducting fibers of the C group. By determining alterations in thresholds of the two responses one may secure an indication of the functional status of the two groups of fibers.

The effects of tetracaine and procaine on thresholds of fast and slow pain were determined on 6 adult, white, male subjects in the manner described above. The results of a representative experiment with procaine are shown in Fig. 1a, and its pair with tetracaine in Fig. 1b. It is apparent that under procaine recovery of fast pain occurred much sooner than that of slow pain. Conversely, under tetracaine, recovery of slow pain took place much earlier with respect to fast pain. In other words, tetracaine had relatively much less effect on fibers mediating slow pain with respect to its action on fast pain than was the case with procaine. Fig. 2 presents the results of all experiments and shows that the same trend was present throughout with

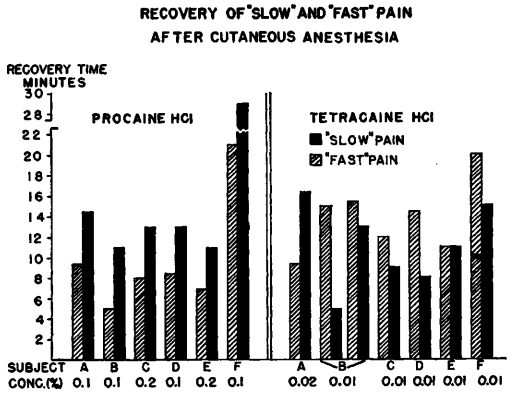

FIG. 2. Results of all experiments on the effects of procaine and tetracaine on slow and fast pain. In the experiment on subject F with tetracaine, there was apparent initial recovery of fast pain at 10 min.; threshold again rose and did not recover until 20 min.

the exception of one test with tetracaine. These differences between the 2 agents are statistically significant to within the 5% confidence limit when calculated by either of 2 methods described above. Determinations of thresholds under tetracaine were more difficult than under procaine, because of the itching produced by tetracaine. This may have contributed towards the lesser regularity of the tetracaine results.

Discussion. The results obtained suggest a reasonable explanation for the paradoxical tetracaine flare. First, as previously recognized(10,11), tetracaine is an irritant to tissue and thus satisfies a requisite for initiation of the flare. Whether tetracaine releases histamine locally or whether it may be intrinsically histamine-like is unknown. When the agent is injected intradermally, the central portion of the injection site may be presumed to have the highest concentration, so that gross testing at this point may reveal clinically complete anesthesia. Toward the periphery of the injection wheal, however, particularly as lateral diffusion takes place, the effective concentration falls off. Thus, at the periphery, the concentration may be high enough to initiate the flare and yet not be high enough to block conduction in the fibers which mediate the response.

The facts here demonstrated, that tetracaine has a lesser effect than procaine on C fibers and that procaine blocks the flare, strongly suggest that the flare is mediated over C fibers. The findings throw no light on the question of further identity of the flare fibers, however. Granting that they are probably of the C group, the two alternatives remain: that they are of the known afferent group of posterior root fibers, or that they are of a separate system, the "nocifensor nerves" of Lewis. The present study offers no evidence for a selection between these alternatives. It is tempting to speculate on the relationship of the flare fibers to the so-called posterior-root vasodilators.

Finally, the difference in effects of procaine and tetracaine on conduction in different groups of nerve fibers is in itself of interest. That members of this class of local anesthetics may differ in this respect has not been suspected, to our knowledge. The basis for the differences is entirely speculative; a possibility that comes to mind is that a difference in rates of diffusion to cell membranes may exist. Everett and Goodsell(12) reported that procaine may spare a group of C fibers when conduction in isolated nerve is tested. This is reminiscent of our results with tetracaine. The discrepancy with our findings on procaine is at present unaccountable.

Summary. 1. Tetracaine hydrochloride, injected intradermally, produces a histamine-like flare which can be blocked by procaine hydrochloride. 2. On the isolated nerve, tetracaine has much less effect on conduction in C fibers with respect to its effect on A fibers than is the case with procaine. (Preliminary experiments.) 3. In the human skin, tetracaine has much less effect on the threshold for "slow" pain with respect to its effect on "fast" pain than is the case with procaine. 4. These observations suggest that the flare is mediated over C fibers. Tetracaine produces a flare 1) by causing irritation, 2) by failing to block completely the C fibers. 5. The differences in action on conduction of tetracaine and procaine are noted, but no explanation for these differences is available.

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