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"SENSITIZATION" BY INJURY OF THE CUTANEOUS NERVE ENDINGS IN THE FROG

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THE present investigation deals with the sensory discharge in the cutaneous nerves of the frog. Its object was to gain further information about the nature of the end-organs and the nerve fibres which give rise to pain. In the frog the pain fibres are generally supposed to belong to the slow group, conducting at 1·5–4·5 m. per sec., for impulses in these fibres are not set up by tactile stimuli, but only by those which would be likely to damage the skin [Adrian, 1931]. The evolution of the discharge resembles the evolution of the pain sensation in man when the skin is injured; for instance, Hogg [1935] finds a long latency and a slow rise and decline in the impulse frequency. But there is another feature of the pain mechanism in human skin which might be looked for in the impulse discharge, namely the lowering of the threshold of the endings to mechanical stimulation after an injury. Lewis & Hess [1933] have studied the "susceptible state" which develops in the human pain endings after injury, and attribute it to the action of a substance liberated from the injured tissues. We have looked for a similar condition produced in the frog's skin by injury, in the hope that, if it were found, it might be possible to identify the substance responsible for the "susceptible state" in this animal. As will be seen, we have found that injury does lower the threshold of the endings of the slow fibres to mechanical stimulation, but we have been unable to identify a substance as the causative factor. In the course of the investigation we have studied the effects of solutions containing various ions in abnormal amount: some of these will be discussed, as they throw additional light on the differences between the endings of the slow and fast fibres.

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Method

Dorsal cutaneous skin nerve preparations were used from both *Rana temporaria* (English) and *Rana esculenta* (Hungarian) frogs. At times, to preserve the cutaneous blood supply, the skin was left attached to the frog. The preparations were either spread out smoothly over a flat ebonite surface through a slit in which the nerve extended to the electrodes in a moist chamber, or were stretched out on the floor of a wax chamber, the nerve leading to the electrodes through a small hole in the floor. The electrodes consisted of cotton wool dipping into U-tubes of Ringer's fluid, which were connected by silver wires, coated with silver chloride, to the input of the amplifying system. This was of the usual type, with recording by loud-speaker, Matthews' oscillograph, viewing screen and camera.

Pressure was applied to the skin by means of a lever which could be loaded with different weights. The stimulating rod which came in contact with the skin was 3 mm. in diameter and its impact was controlled by an oil dashpot. Injury to the skin was produced by scraping or pressure as described later. Tissue "juice" was obtained by scraping the skin of the frog's legs with a scalpel, the moist scrapings being collected on a small piece of filter paper. Solutions used were:

(1) Ringer: NaCl 0·65 p.c.; $NaHCO_3$ 0·015 p.c.; KCl 0·02 p.c.; $CaCl_2$ 0·025 p.c.

(2) KCl solutions: Ringer with KCl content raised to 0·2 p.c.

(3) $CaCl_2$ solutions: Ringer with $CaCl_2$ content raised to 0·05–0·25 p.c.

(4) NaCl solutions: Ringer with NaCl raised to 1·3, 1·62 and 1·95 p.c.

(5) Glucose solutions: Ringer containing 3–10 p.c. $C_6H_{12}O_6$.

Results

Sensitization by injury

In assessing the effects of injury on the cutaneous nerve endings, the most important factor is to ensure that the same endings are exposed to the same intensity of stimulation, before and after the skin has been injured. To comply with this requirement as nearly as possible, we have used a small glass stimulating rod, attached to a lever which could be loaded with varying weights. The stimulating surface of the glass rod was 3 mm. in diameter and could be applied to a selected spot on the skin and the resulting discharge to a fixed pressure observed. The rod could then be withdrawn without moving the position of the lever, so that after

injuring the skin the rod, on release, would come to rest again with the fixed degree of pressure on the same spot as before.

The skin was injured by very gently scraping the surface with a scalpel in the region where the rod had been resting. This type of injury abolishes or diminishes the response of the tactile system (fast impulses), and if not too severe leaves the slow impulse system intact [Adrian, 1931; Feng, 1933]. Injury was also applied by temporarily increasing the pressure, through the stimulating rod, to 30–50 g. or more. This type of injury also abolishes the fast impulse response, but leaves the slow one unimpaired if the pressure is not too great. The pressure was usually applied interruptedly by withdrawing and releasing the stimulating rod

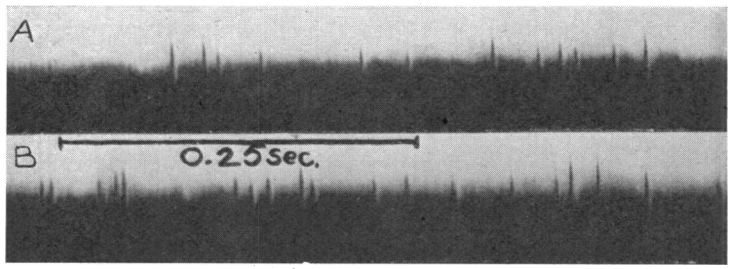

Fig. 1. Slow impulse response to 20 g. pressure applied to the same spot on the skin for 5 sec. A before and B after injury of the skin by gentle scraping. Note that the frequency of the slow impulses is increased after injury.

every 4–5 sec. while observing the discharge in the viewing screen. This procedure gave an opportunity of testing the impulse response at frequent intervals.

Our preliminary experiments showed an apparent increase in the slow impulse discharge after injury. A series of observations was accordingly made with the conditions standardized as far as possible.

Pressures of 10, 15 g., or more, were applied to a selected spot in the receptive field of the skin and the impulse discharge photographed at intervals. After injuring the skin in the manner described, the impulse responses from similar pressures applied to the same area were photographed at corresponding intervals. Some increase in the slow impulse discharge to a given pressure was found in twenty preparations, but in about as many others no definite increase was present, and in a few preparations it was diminished. The increase was never great, and in those preparations regarded as showing it, the discharge, after injury, had a frequency of $1\frac{1}{2}$–4 times that in the uninjured skin (see Fig. 1). In the majority of this group the frequency was doubled after injury.

Although these experiments showed some increase in the response of the slow endings, it was clear that, for proof of a lowering of their threshold, test stimuli of just subthreshold value for the slow endings would have to be used in the uninjured skin; for pressures of 10 g. and over which had been employed were well above the slow impulse threshold.

In a second series of experiments, therefore, the threshold was found in the uninjured skin by observing the discharge in the viewing screen. This varied slightly in different preparations and in different areas in the same preparation, and was usually considerably below 2·0 g. per sq. mm., the value given by Hogg [1935].[1] The just subthreshold degree of pressure for a given area was then determined as nearly as possible and used as the test stimulus. The skin was injured and the same procedure followed as

TABLE I. Frequency of slow impulse discharge per sec.

Prep.	Duration of pressure	Before injury	After injury
1	Onset	3	5
	1 sec.	4	8
	5 ,,	3	10
	15 ,,	2	8
2	Onset	0	8
	1 sec.	1	15
	5 ,,	0	13
	15 ,,	1	14
3	Onset	0	5
	1 sec.	3	8
	2 ,,	3	10
4	Onset	2	8
	1 sec.	2	11
	5 ,,	5	14
5	Onset	0	2
	1 sec.	0	4
	5 ,,	0	8
6	Onset	0	1
	1 sec.	1	2
	3 ,,	0	11
	5 ,,	0	8
7	Onset	1	13
	1 sec.	3	17
	5 ,,	5	17
	15 ,,	3	16
8	Onset	2	8
	2 sec.	4	12
	5 ,,	7	30
9	Onset	3	9
	5 sec.	5	16
10	Onset	0	5
	1 sec.	4	8
	3 ,,	3	11
11	Onset	2	7
	5 sec.	3	15
	15 ,,	1	15
12	Onset	0	4
	2 sec.	2	8
	5 ,,	1	12
13	Onset	3	3
	5 sec.	3	7
	15 ,,	1	6
14	2 ,,	0	10
	5 ,,	0	9
	15 ,,	0	11
15	5 ,,	5	12
16	5 ,,	5	20
17	4 ,,	3	10
18	5 ,,	3	19
19	5 ,,	4	22
20	5 ,,	4	13
21	5 ,,	3	10
22	5 ,,	6	10
23	5 ,,	0	14
24	5 ,,	4	12
25	5 ,,	3	20
26	5 ,,	5	22

[1] In fact values as low as 0·25 g. per sq. mm. have been obtained. This marked variance in the threshold value found by Hogg and ourselves may be due to the difference in the surface area of the stimulating objects used. We have not determined accurately the threshold of the fast fibre endings but they respond to the touch of a fine hair.

already described, photographs being taken of the resulting discharge to the test stimulus at given intervals before and after injury. After injury time was allowed for the spontaneous slow impulse discharge, when present, to subside, and a photograph of the base-line was taken to confirm this, before again photographing the response to the test stimulus.

In every preparation (30) in this series the test stimulus before injury produced only an occasional slow impulse, or none at all, whereas after

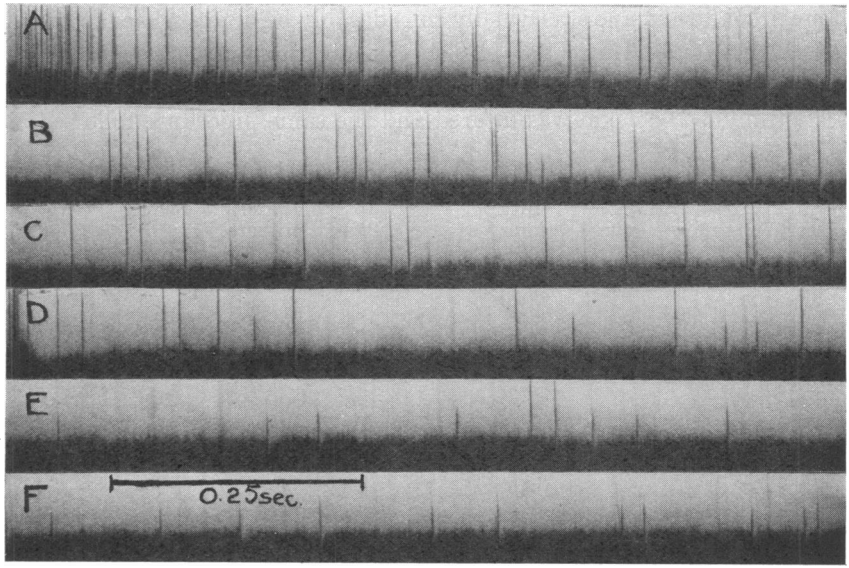

Fig. 2. Showing the effect of injury on the response of the cutaneous nerve endings to a constant pressure of 2 g. A, B, C, before and D, E, F, after injury of the skin by gentle scraping. A, at onset of pressure; B, after pressure applied for 2 sec. C, pressure applied for 5 sec. D, E, F, at onset of pressure to same area, and at 2 and 5 sec. respectively. Note that after injury (D, E, F) the slow impulse response is increased and the fast diminished. The latent period to maximum frequency of the slow impulse discharge is evident, being highest in plate F at 5 sec.

injury the discharge was abundant, considering the small degree of pressure used and the usual low frequency in the slow fibres (see Table I and Figs. 2, 3, 4). At the same time, injury temporarily diminished the fast impulse reponse and if severe enough abolished it.

In certain areas of a few preparations, although the test stimulus gave rise to fast impulses, very few slow ones were obtained either before or after injury. In other areas of these preparations, however, the usual "sensitization" of the slow endings resulted from injury. This discrepancy

could easily be explained on the basis of an uneven distribution of the slow endings in the skin. Also in a few areas in several preparations although the test stimulus produced a few slow impulses, no increase was found to the same stimulus after injury. We believe that these latter results can be attributed to too severe injury putting permanently out of action some of the endings. When less injury was used it frequently

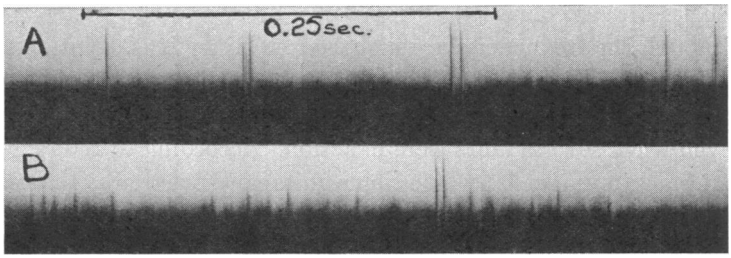

Fig. 3. Demonstrates that the threshold to pressure of the slow fibre endings is lowered by injury. A, response from uninjured skin subjected to a pressure of 2 g. for 5 sec., showing only fast impulses. B, response from same area to 2 g. pressure for 5 sec., after injury of the skin by scraping. Abundant slow impulses are now present.

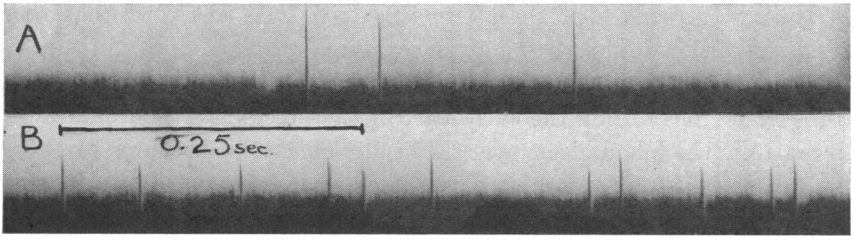

Fig. 4. Impulse response to 2 g. pressure applied to the same spot on the skin for 15 sec. A, before and B, after injury of the skin by gentle scraping. Demonstrates that the threshold to pressure of the slow fibre endings is lowered by injury, since slow impulses are present only in B.

happened that only a feeble discharge of slow impulses was obtained until several minutes had elapsed, when it became abundant. This interval, although it could be the result of the gradual action of some "sensitizing" substance, has seemed more likely to be due to the slow recovery of the endings from trauma since it is not usually evident if the injury is still less severe. These latter effects, as well as "sensitization" of the slow endings, have been more easy to demonstrate in Hungarian than in English frogs, since injury is less likely to destroy their nerve endings.

As Hogg found, there is a considerable latent period after pressure is applied before the frequency of the slow impulse discharge reaches its maximum [Hogg, 1935]. With the lighter pressures used (under 2·5 g.) this latency has been as long as 5 sec., but with heavier pressures (15–20 g.) it has usually been about 0·75 sec. [Hogg, 1935]. We have not, however, found any appreciable alteration in the length of this latent period to a given pressure before and after injury although, of course, the frequencies are different.

Although the frequencies are higher after injury it is of interest that the highest frequency of slow impulse discharge we have obtained with mechanical stimulation over a surface of 3 mm. in diameter was 60 per sec., even when several units were in action. According to Hogg, 40 per sec. is the maximum rate at which single units can discharge by themselves. As to the adaptation time of these ending we agree with Hogg that it is extremely slow, for on several occasions after injury a pressure of 15 g. has given rise to a discharge of slow impulses, after the first $\frac{1}{2}$ min. that showed little evidence of diminished frequency in half an hour. We also agree with him that the slow impulse discharge "never ceases abruptly on cessation of stimulation", but we find that often only a few scattered impulses occur after withdrawal of the stimulus.

During the course of these experiments it was observed that the discharge of slow impulses to a given pressure for a fixed duration varied widely in frogs of the same type at different periods. For instance, in Hungarian frogs during the early part of the breeding season, and in a large batch which had ulcers over the head regions, the discharge reached 40 per sec. with 15 g. pressure in the uninjured preparation. This is about double the frequency obtained in the same type of frogs during other periods.

The effect of potassium on the cutaneous nerve endings

Feng [1933] has considered the possibility that a liberation of potassium might account for some of the sensory effects of injury. He showed that the temporary abolition of the tactile response when the frog's skin is scraped might be due to an excess of K ions; for he found that a reversible abolition of tactile impulses could be produced, without apparent effect on the slow fibre system, by placing injured tissues on the subcutaneous surface of the skin, or exposing it to a fluid containing the concentration of potassium which would be present in the injured tissue (i.e. about ten times the amount in normal Ringer's fluid).

Although Feng had considered and rejected the suggestion that the excess of K might stimulate the slow endings, it seemed possible that it might play some part in their sensitization to mechanical stimulation.

From Feng's account it was not certain that the site of action of the excess potassium was on the nerve endings rather than on the nerve fibres, since the K solutions or tissue scrapings were placed on the subcutaneous side of the skin, where they might come in contact with the nerve. It seemed possible therefore that the slow impulses which can be obtained after treatment of a preparation with potassium solution might be fast ones travelling in a nerve whose conduction had been slowed. We find, however, that 0·2 p.c. K solutions though they abolish the tactile response from the area of skin to which they are applied are without definite effect on the nerve fibres in the cutaneous nerve. The evidence for this comes from experiments in which the nerve was slung across an electrode in which the cotton wool was soaked in Ringer's fluid, containing 0·2 p.c. K (ten times the normal amount). In no case after an interval of 20 min. was there any interference with the conduction of the fast impulses past the electrodes, though the same solution applied to the back of the skin abolished the fast impulses in 5–15 min. Also, when the potassium was restricted to a small area of the back (subcutaneous surface) of the skin, by placing it on swabs of cotton wool, it abolished the fast impulse response in this area, but not from the neighbouring regions. The nerve therefore was conducting normally and the slow impulses which were easily obtained from the area treated with K were genuine ones, and not fast impulses travelling in a slowly conducting nerve. It was further observed that the elevated touch spots were the last to lose their response to touch when the K solution was applied to the whole preparation or to the subcutaneous surface alone. This was often found even when the touch spots were widely scattered over the skin, the intervening areas becoming unresponsive. The reason why they should be affected later than the other endings is possibly that the K would take longer to reach them, since the K, to have effect, must be applied to the subcutaneous surface of the preparation. 0·2 p.c. K solutions restricted to the outer surface of the preparations, where the elevated touch spots are located, had no effect in half an hour. We conclude that the action of the excess K is on the nerve endings, or at any rate on the terminal parts of the nerve fibres rather than on the nerve trunks.

We have confirmed Feng's finding that injured tissue (the scraping of skin from frog's legs) applied to the subcutaneous surface of the skin acts like K solutions in abolishing the tactile response. We have also found, as he did, that neither injured tissue nor K solutions give rise to a spontaneous discharge of slow impulses from the skin. Occasionally a few have appeared, but they were most likely the result of handling the preparation.

On the question of the sensitization of the slow fibre endings, however, we have found it very difficult to obtain conclusive evidence. With pressures of 2 g. per sq. mm., and above, slow impulses are easy to obtain in a preparation treated with potassium, and in a few cases there has been a slight increase in impulse frequency over the untreated preparations. The increase is certainly not as great as with injury however, and we are not convinced that it is outside the range of error. Several difficulties arise in interpreting the results. In the first place, since pressure must be

used to obtain the slow impulses, any increase in the discharge that is observed may be due to the injury produced by the pressure itself. The results are complicated as well by the fact that a solution containing 0·2 p.c. K will abolish even the response of the slow system in about half an hour, so that a sensitizing effect, if its exists, will soon merge into a depression. The preparations in which all response has been abolished in this manner show a recovery of both slow and fast impulse response after an immersion for an hour in normal Ringer's fluid.

Stimulation of the endings of the slow and fast fibres by hypertonic solutions and $CaCl_2$

Although injury is not likely to cause an increase in the osmotic pressure of the tissue fluids sufficient to stimulate the pain endings, it is well known that subcutaneous injections of hypertonic glucose or NaCl

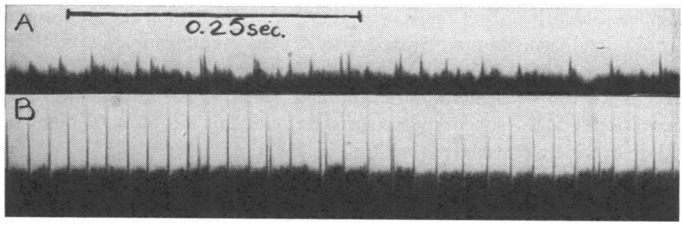

Fig. 5. Shows the impulse response after irrigating the skin with Ringer's fluid containing $2\frac{1}{2}$ × the normal quantity of NaCl. A, response when skin irrigated for 1 min. and is composed entirely of slow impulses. B, spontaneous rhythmical outburst of fast impulses which occurred after 20 min. irrigation.

solutions will cause pain. We have therefore investigated the effects of 4–10 p.c. glucose and of 1·3–1·95 p.c. NaCl solutions on the frog's skin.

Preparations irrigated or immersed in Ringer's fluid containing 4–10 p.c. glucose gave a persistent spontaneous discharge of slow impulses, which came on within $\frac{1}{2}$–2 min. and showed only a slightly diminished frequency during the following hour. Fast impulses appeared after about 5 min., but remained infrequent except with 7·5–10 p.c. glucose solutions. Even with these concentrations the fast impulse discharge remained irregular except for a few short rhythmical bursts.

In preparations treated with NaCl 1·3–1·95 p.c. Ringer's fluid, however, although they showed the same type of spontaneous slow impulse discharge (see Fig. 5A), long rhythmical discharges of fast impulses appeared on touching the skin with a glass rod in 15–20 min. after irrigation (see Fig. 5B). Since the tendency to rhythmical impulse discharges

is much less marked in preparations treated with glucose solutions of the same osmotic pressure, this effect of the NaCl is evidently due to the disturbance of the Na/Ca balance. Talaat [1933] found a similar type of rhythmical after-discharge in preparations bathed in Ca free Ringer's solution (0·65 p.c. NaCl) and this would disappear if Ca were added to the irrigating fluid.

Since Talaat [1933] also found that removing the Ca from the Ringer's fluid bathing a preparation would cause a lengthening in the adaptation time of the sensory receptors, it seemed interesting to try the effect of increasing the calcium content of the Ringer's fluid above the normal

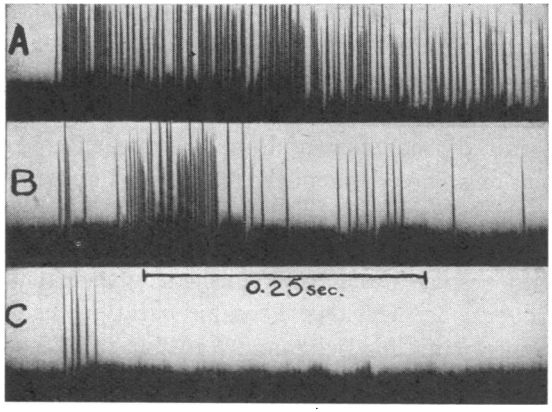

Fig. 6. Shows the impulse response to 10 g. pressure on the skin before and after irrigation with Ringer's fluid containing 0·25 p.c. $CaCl_2$. A, response to pressure in normal skin. B, response to pressure over an elevated touch spot after 10 min. irrigation. C, response to pressure over the smooth portion of the skin, taken just before B.

value. Frogs, during the breeding season and for a considerable period after, are very suitable for a study of this kind, as the endings of the fast fibres adapt quite slowly to a constant pressure. For instance, a constant pressure of 10 g. causes an outburst of fast impulses, which maintains a high but diminishing frequency for from 5 to 15 sec., thereafter becoming irregular and diminishing rapidly in rate. This type of discharge was photographed in normal preparations (see Fig. 6A) and after immersing the preparations in or irrigating their subcutaneous surface with 0·05–0·25 p.c. $CaCl_2$ (twice to ten times the normal amount in Ringer's fluid). The most obvious result of the treatment was that the receptors of the fast fibres became much more rapidly adapted to the pressure: this is in agreement with Matthews' finding [1931] that if $CaCl_2$ is added to a

NaCl solution bathing a frog's muscle spindle the adaptation time to stretch becomes shortened. After irrigation with 0·25 p.c. $CaCl_2$ (ten times the amount in normal Ringer's fluid) for 10 min., although the preparations' sensitivity to light touch was not obviously diminished, the discharge to pressure was reduced to one that was practically over in less than a second (see Fig. 6C). A similar effect was obtained with 0·05 p.c. $CaCl_2$ chloride, but it took much longer to be established, the preparations usually being immersed in the fluid and placed in the ice box for several hours. With the higher concentrations the effect of the calcium was easily restricted to a small area of the preparation as described with potassium. Again, the last areas to show a shortening of discharge were the elevated touch spots. This was quite striking, for often pressure anywhere gave only a momentary outburst of impulses until, coming in contact with a touch spot, a discharge of considerable duration occurred (see Fig. 6B). Apart from a shortening of the discharge the $CaCl_2$ solutions also diminished the initial frequency and, in the higher concentrations, if allowed to act long enough, abolished the fast impulses. After these had gone, pressure still produced a discharge in the slow fibres, which did not appear shorter than in the normal preparation. In time, however, the slow impulse response was also abolished by the calcium solution if used in the higher concentrations. These preparations usually recovered if placed in Ringer's fluid for 1–2 hours. $CaCl_2$ up to 0·25 p.c. in Ringer's solution, like K, never produced a spontaneous discharge, and if placed on the outer surface of the preparation had no effect on the response to pressure. Replacing the Ringer's fluid in the first electrode with Ringer's containing 0·25 p.c. $CaCl_2$, and leaving the nerve in contact with this for 20 min., had no apparent effect on the discharge from stimulating the skin.

In Hungarian frogs a peculiar effect was noted, and it is here mentioned so that others may not be misled, as we were at first. If a moist material of apparently any nature is placed on the surface of the skin of these frogs a spontaneous discharge of fast impulses results. It occurs with cotton wool soaked in water, Ringer's fluid, Ringer's fluid plus 0·2 p.c. K, or 0·25 p.c. $CaCl_2$, as well as with tissue scrapings from the frog's skin. The spontaneous discharge comes on slowly, a few fast impulses occurring only after about 5 min. These gradually increase in frequency, reaching their maximum (94 per sec.) at 15–20 min. but always remaining irregular. Only a very few slow impulses appear, but the fast continue undiminished as long as the stimulating material is present. When it is removed the discharge subsides only gradually, taking 2–5 min. to disappear. The

preparation is then found to be normally sensitive even after an hour of this treatment. The same phenomena were observed when the skin from these frogs was immersed in Ringer's fluid (in a wax chamber). From the nature of the discharge it seems probable that it is due to some mechanical disturbance, but we are unable to explain it.

Discussion

It has been found that the endings of the slow conducting fibres in the frog's skin show a lowering of their threshold or an increase in response to mechanical stimulation following injury. Human pain endings also become "hypersensitive" after injury, but this is no proof that their function is in any way similar to the endings of the slow fibres in the frog. The finding is, however, not out of keeping with the supposition that the frog's slow fibre system is concerned with pain, and if the supposition is correct it suggests another point of similarity between the frog's and the human pain mechanism.

It is interesting to consider together the effect on the frog's skin of injury, tissue scrapings and potassium. Either one will abolish the response of the fast impulses (response to light touch) before the slow ones (response to pressure), and, as Feng has shown, it is apparently potassium that is responsible in the tissue scrapings. Again, if one examines the effect of injury on the human skin by pressing a hard object (the wooden end of a match) firmly against the back of the hand for several minutes until pain results, one finds that the sensation to light touch has become diminished in the area pressed upon. Whether the mechanism is similar in the two cases and whether potassium is responsible we are unable to say.

With regard to the role of ions in stimulating the endings of the slow fibres to discharge, there is no evidence that this occurs under ordinary circumstances in the frog. The sodium calcium ratio probably plays a role in normal adaptation but it is unlikely that the calcium would ever be reduced or the sodium raised sufficiently to cause an impulse discharge. There is still the possibility that potassium is concerned in the "sensitizing" of the slow fibre endings by injury, but we have been unable to find conclusive evidence of this.

Summary and conclusions

1. The effect of injury, potassium, calcium and hypertonic sodium chloride and glucose on the cutaneous nerve endings in the frog have been studied.

2. Pressure on the frog's skin, like scraping, has a differential action on the various nerve endings, abolishing the response of the receptors for light touch (fast impulses) before those responding to pressure (slow impulses).

3. Injury to the frog's skin either by pressure or gentle scraping causes a lowering of the threshold or an increase in response to mechanical stimulation of the endings of the slow conducting fibres.

4. These endings, like the pain endings in the human skin, are therefore "hypersensitive" following injury.

5. The action of potassium chloride in abolishing the response to touch in the frog's skin is on the terminal nerve fibres or nerve endings.

6. The touch receptors in the frog's skin adapt more rapidly after treatment with calcium chloride and its action is on the terminal nerve fibres or nerve endings.

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