A PRELIMINARY STUDY ON THE EFFECTS OF SUPERFICIAL APPLICATION OF SALT SOLUTION ON LIVING FROGS

By

B. P. CHAKRAVORTI

Grant Medical College, Bombay.

(Received March, 28, 1958)

The effects of various salts on the isolated organs of frogs are known; but very little information is available regarding the behaviour of an intact living frog, when brought in contact with different salt solutions. The present paper deals with the study of the phenomenon of change of colour of frogs in various salt solutions. The investigation has some bearing on the idea suggested by Chatterjee (1956) that the disturbance of ionic balance may alter the activity of enzyme melanase leading to the excess melanin formation, while commenting on the divergent theories to explain the bronzing of the skin due to the deposition of melanin in Addison's disease in man. Chatterjee has however not produced any evidence to substantiate his view but has obviously been led to this conclusion from the generally known principle that the inorganic ions in many places guide the enzyme action.

Hogben and Winton (1922) studied the change of colour of the skin of frog and explained the mechanism on the basis of hormonal secretion. They showed that pituitrin causes the pigmenting granules in the melanophores to become dispersed throughout the body and the branching of the cells, resulting in the darkening of the skin. Thus the injection of a drop of a solution of pituitarin caused the skin to become almost coal black. Adrenaline has the opposite effect. Stoppani (1942) has demonstrated that when 0.5 mg. of adrenaline is injected into dark toads they turned pale in 80 minutes. The accepted view so far is that the change of colour in amphibia to blend into the colour of the surrounding is largely due to the variation in concentration of the pituitrin in blood or to the balance between the concentration of the melanophore expanding principles, and adrenaline (Houssay *et al.*, 1955). Another factor which influences the granules of the pigment cells of the skin is the nerves acting on the pigment cells (Best and Taylor 1950).

If Chatterjee's view about the pigmentation in Addison's disease in man is correct, it seems logical to expect that the colour change of the skin of frog may also be due to the ionic imbalance. Another reason which induced the author to take up this work is to find out an explanation for the interesting phenomenon, commonly observed in this part of the country that in the rainy season, most of the frogs are of pale colour, whereas in other seasons majority are of dark colour. In the rainy season the concentration of the salts in the habitats of frogs become greatly diluted and it seems probable, therefore that the change in concentration or the osmotic tension of the salts may have some influence on the colour change of the frogs.

METHODS

The species of frog chosen for the present study was *Ranatigrina* and specially the ones having the yellow colouration of the skin were selected. Saturated solutions of different salts, namely, sodium chloride, potassium chloride, and calcium chloride were prepared. Ten frogs of above mentioned variety having nearly the same weight, sex, and same intensity of yellow colour of their skin were selected for each set of experiment with the above mentioned salts. They were all secured by strings tied at their groin to keep them in control. A set of five frogs was kept for comparison as control without any treatment with these salts. Small pieces of cloth (2″ by 1″) were moistened with different salt solutions, viz., saturated solutions of sodium chloride, potassium chloride, and calcium chloride separately and each was kept on the yellow area on the back of the individual frog for duration of five minutes. After this period, the cloth piece was removed from the surface of the skin. These frogs together with the untreated control frogs were kept in a sink under the slowly running tap water.

RESULTS

The colour of the area of the skin, where the saturated solution of calcium chloride was applied turned from yellow to Hooker's green and that of the frog treated with potassium chloride and sodium chloride turned black green in colour. When they were all kept together under a slowly running tap water for three hours they changed their colours to dark black. The colour of untreated control frogs (five were used for control under the same condition except that these were not treated with salts) did not change at all.

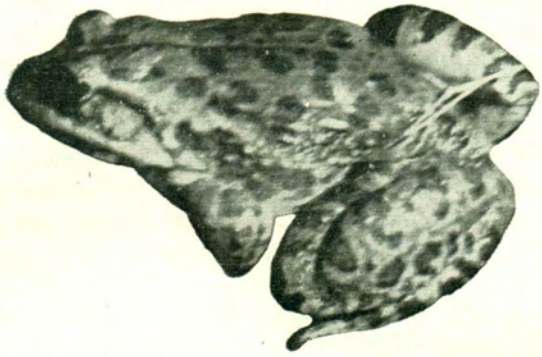

FIG. 1. Normal frog., Without any application of salt solution on the skin. The original colour of the skin was yellow.

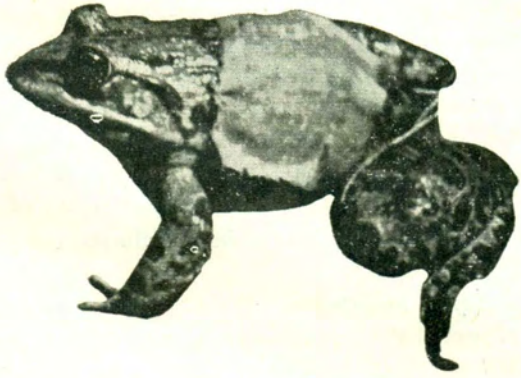

FIG. 2. The method of application of the salt solution on the skin of above frog. Solution of the salts were applied on the cloth shown in the fig.

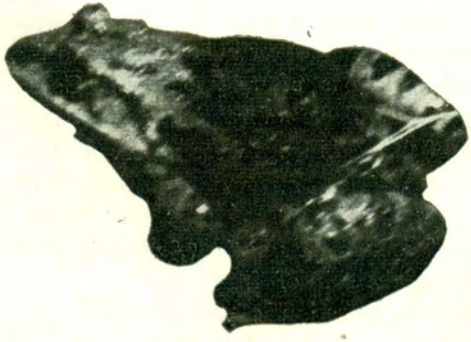

FIG. 3. Darkening of the area of the skin of the above frog., when the solution of salt was applied on the back-skin. This darkening spread gradually throughout the body specially on the dorsal aspect of the animal.

The change of colour took place throughout the whole body though the salts were applied only on a certain area of the skin. The colour diffused on the entire dorsal epidermal region overnight, and persisted for more than 48 hours.

No change of colour was noticed on the ventral aspect of the frog except that in case of calcium chloride treated frogs, a light red patch was observed specially on the abdomen and the legs. The red patch reduced after 5 hrs. and completely disappeared overnight.

Regarding the general behaviour, immediately after the application of the salts, the frogs appeared restless but showed the normal behaviour after an hour.

Effects of different concentration of the salts. To eliminate the possible irritable effects of saturated solutions of the salts, the same salts in lower concentrations were also used. One percent, 5 percent, 10 percent, and 20 percent, solutions of the salts mentioned above, viz., sodium chloride, potassium chloride, and calcium chloride, were applied and experiments were conducted in the same manner as described above, on a set of five frogs in each concentration of each salt. Change of colour from original yellow to dark black was similar to that observed in saturated solution. General behaviour of the frogs did not indicate any sign of irritability. The animals behaved quite normally.

Effects of the salts after decerebration. If the pituitary and the nerve theories are correct, it is expected that no reaction in respect of change of colour will be possible if the nervous system is destroyed. To verify this, five frogs were decerebrated, and the saturated solutions of above mentioned salts were applied on the still living frogs and expriment was carried out in the same manner as described earlier. The frogs died after $1\frac{1}{2}$ to 2 hrs. The change of colour from yellow to black occured as in case of similar experiments on intact frogs, the intensity of the colour being similar to that observed in cases of experiments with intact frogs after $1\frac{1}{2}$ to 2 hrs. Colour disappeared after the death of the frogs. The effects of application of the saturated solution of the salts on the skin of the dead frogs were observed to have no effect regarding the change of colour.

Effects of addition of salts to the secretion of the skin of frog. Assuming that bufotoxin, a secretion of the skin of frog may have some thing to do in the colour change of the skin, secretion of the skin was collected and allowed to react with salts in vitro; no change of colour was observed.

CONCLUSION

It will appear from the above experiments that the effects of the salts in producing the change of colour from yellow to black is very efficient and rapid. The reaction of the salts appears to be first localised and then dispersed and the effects last for some days. When the nervous system is destroyed, similar change in colour as in case of intact frogs is also observed. Bufotoxin has nothing to do with the colour change.

DISCUSSION

The present studies though preliminary, lead one to think that the hormone, or the nervous theory may not fully explain the mechanism of the blackening of the skin of the frogs. The pituitrin theory of Hogben and Winton seems to fail apparently, because the black pigmentation can be produced even on a decerebrated frog. The nervous theory also similarly falls on the same ground. This investigation though apparently supports Chatterjee's viewpoint does not however necessarily go against the hormone theory, for it seems likely that the hormone may not be responsible in a direct way as is generally believed, but works in an indirect manner causing the ionic imbalance of the system which in its turn is probably affecting the enzymatic system responsible for the change of colour. Though still premature for generalisation, the investigation seems to lead to a very important expectation that the mechanism of the hormone reaction may not be direct as it is believed to day, but it seems that hormones work indirectly by influencing the enzymatic system which is possibly responsible for the alteration of all cellular mechanism through ionic imbalance.

SUMMARY

1. Effects of hypertonic solutions of salts, e. g., calcium chloride, potassium chloride and sodium chloride on the living frogs have been described.

2. The change of colour from yellow to dark black of the skin of frog has been mentioned.

3. Photograph of frogs treated with different salts are represented.

ACKNOWLEDGEMENT

I am thankful to the head of the Dept. of Physiology, Grant Medical College, Bombay for allowing me to carry on this work. I am thankful to Mr. Y. B. Gupte for helping me in taking the photographs represented here.

REFERENCES

1. Chatterjee, C. C. (1956): *Human Physiology. 3rd edition. Book concern, page 499.*
2. Hogben, L. T., and Winton, F. R., (1922): *Proc. Royl. So. Biol.*, **318**, *93.*
3. Stoppani, A., (1942): *Endocrinology*; **782,** *30.*
4. Houssay, B. A., and others, (1955): *Human Physiology, 2nd edition, McGraw Hill Book Comp. I. N. C. pages 574-576.*
5. Best, C.H., and Taylor, N.B. (1950) : *Physiological Basis of Medical Practice. 5th edition, William and Wilkins Camp. Baltimore, page. 849-850.*