

of the suprarenal cortex which is so strikingly beneficial in Graves' disease. It is possible, however, that parenteral administration of hexuronic acid may be effective.

This evidence strongly supports the conclusion reported in our previous papers that (1) a symptom-complex essentially identical with Graves' disease, in its physiological and pathological aspects, can be produced by sublethal injury of the suprarenal cortex of rabbits and cats;^{4, 5} (2) a glycerol extract of suprarenal cortex consistently causes strikingly beneficial effects in Graves' disease; (3) this suprarenal cortex extract as shown in 1926⁶ lowers the metabolism of rabbits when thyroids are intact and (4) when hexuronic acid from suprarenal cortex is substituted for the glycerol extract of suprarenal cortex, no beneficial effects occur. We are convinced that the primary etiological factor in Graves' disease is the deficiency of the internal secretion of the suprarenal cortex.*

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On the Nature of the Dye Penetrating *Nitella* from Cresyl Blue.

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(1) Measurements of the sap of *Nitella B* with the glass electrode made with actively growing cells collected in early summer confirmed those formerly obtained^{1, 2} and showed that there were no seasonal changes affecting the results. Living cells were placed in cresyl blue solution at pH 9 until 0.07% dye had penetrated the vacuole, causing an increase of about 0.4 pH in the sap. This corresponded to the increase when 0.07% dye base was dissolved in the sap *in vitro*, but not to the behavior of the dye salt which did not raise the pH value. These results show that the dye does not penetrate as a salt (undissociated DCl or $D^+ + Cl^-$).

⁴ Marine, D., and Baumann, Emil J., *Am. J. Physiol.*, 1922, **59**, 353.

⁵ Scott, W. J. M., *J. Exp. Med.*, 1922, **36**, 199.

⁶ Marine, D., Baumann, Emil J., and Cipra, A., *Am. J. Physiol.*, 1925, **72**, 248.

* There is abundant evidence that the gonads contain a similar substance.

¹ Through the kindness of Dr. D. A. MacInnes and Dr. M. Dole the applicability of the glass electrode method to the studies of single cells was tested. *J. Gen. Physiol.*, 1928-29, **12**, 805.

² Irwin, M., *Proc. Soc. Exp. Biol. and Med.*, 1929, **27**, 132; *J. Gen. Physiol.*, 1930-31, **14**, 1.

They also show that in case *D* ions penetrated by an exchange of cations, they must have exchanged with H ions to account for the increase in the pH value of the sap. In this case more H ions must diffuse than K ions. But this is very improbable, owing to the fact that the concentration of H ions is several thousand times lower than that of the K ions in the sap while there is no indication that the mobility of the H ions in the protoplasm is very different from that of the K ions. It must therefore be concluded that the dye penetrates as a base. Whether it does so in an undissociated form or as an ion pair must be left undecided.

(2) If the dye penetrates as *D* ions we should expect their mobility in the protoplasm to be very high since the dye penetrates rapidly even from a very dilute solution. This was tested by measurements of potential difference on living cells, employing 0.01 M CaCl_2 and cresyl blue at pH 8 and pH 9 made up in diluted borate buffer mixtures containing no K. The pH values were checked by means of the glass electrode. Owing to uncertainty as to the absolute concentration of cresyl blue, several concentrations were used against 0.01 M CaCl_2 and were found to give approximately the same readings. The results show that the mobility of *D* ions is about the same as that of Ca ions which is considered by Osterhout³ to be very low. They therefore tend to favor the idea of penetration as the undissociated base.

(3) To understand the rôle of this dye base it is necessary to consider the various components of cresyl blue solution. In the experiments on *Nitella*, roughly between pH 6 and pH 9, the external solution gives an absorption curve identical with cresyl blue and remains unaltered. But if the cresyl blue solution at a pH value above pH 8 is shaken with benzene or chloroform until a great deal of the blue dye (giving an absorption spectrum identical with cresyl blue) is absorbed, the aqueous solution appears to contain a mixture of cresyl blue and a violet dye formed irreversibly. The amount of the violet dye is so small that it does not seriously affect the determination of the partition coefficients if sufficient quantity of the blue dye is left in the aqueous solution at equilibrium (as in former experiments) nor does it affect the experiments on *Nitella*. Other irreversibly formed substances at higher pH values appear too far outside the range to play any part in the experiments on *Nitella*. The same may be said of the oxazon⁴ which is formed below pH 4 and which is not present in the sample employed. At

³ Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715.

⁴ Cohen, B., and Preisler, P. W., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 92.

about pH 11 the dye becomes somewhat orange but on immediate addition of acid the blue color reappears. This orange dye may be an imine base: it decomposes rapidly.

The dissociation constant⁵ of the dye base which penetrates is about $10^{-5.6}$ as shown by the experiments on *Nitella* and on partition coefficients. This dye is spectrophotometrically² identical with cresyl blue but the name may be altered to cresyl blue X since this dye is regarded by Cohen⁴ and Preisler (on the basis of oxidation-reduction potential measurements) as a decomposition product of cresyl blue while cresyl blue is regarded by them as more strongly basic. But this does not alter any of the results and conclusions heretofore obtained. On this basis a parallel may exist in the case of methylene blue⁶ from which also the more weakly basic component (azure B) penetrates more rapidly than methylene blue.

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Effects of Posterior Pituitary Extracts on the Lactic Acid of the Blood.

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It has been noted that pitocin increased the metabolic rate of rats, while pituitrin and pitressin decreased the oxygen consumption.¹ In the present experiments the effects of 20 to 30 cc.* of chloretone-free solution of each of the 3 posterior pituitary extracts were observed on 3 unanesthetized and 2 amytalized dogs. Subcutaneous injections were made at half hourly intervals and blood samples drawn every 2 hours for 6 hours were analyzed for lactic acid.

The 3 unanesthetized animals that received pitressin showed a consistent increase in lactic acid so that the results of any one of these may be taken as representative. In each of the 3 experiments, by the end of the second hour lactic acid had increased at least 20 mg. per cent, and then gradually diminished until the sixth

⁵ Irwin, M., *J. Gen. Physiol.*, 1925-26, **9**, 561.

⁶ Irwin, M., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 563; *J. Gen. Physiol.*, 1928-29, **12**, 147; 1930-31, **14**, 19.

¹ Himwich, H. E., and Haynes, F. W., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 815.

* 1 cc. pituitrin contains 10 oxytocic and 10 pressor units. 1 cc. pitocin contains 10 oxytocic units and 1 cc. pitressin contains 10 pressor units.