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Effects of Intraventricular Injections of Pituitrin and Pilocarpine in Dogs.

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(Introduced by Harvey Cushing.)

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Cushing[1] recently showed that the "active principle of the neurohypophysis—pituitrin, when introduced into the ventricle, causes flushing, sweating, salivation, lachrymation, vomiting and pronounced fall in body temperature." This was interpreted as essentially a cranial autonomic or parasympathetic effect, analogous to the reaction on the sympathetic system produced by a discharge of adrenalin into the blood stream. He also found that pilocarpine injected into the ventricle produced essentially the same effect as pituitrin, although less pronounced. When atropine was injected into the ventricle a few minutes before either of the other drugs, or when the patient was under avertin anesthesia, the above reactions did not take place. At Dr. Cushing's suggestion an attempt has been made to compare the reactions thus obtained on human subjects with those of laboratory animals. The present report is based on the results of such studies carried out on dogs.

Methods. In some of the early experiments the animals were under morphia and intravenous chloralose or morphia and ether throughout the experiment. Most of the experiments were carried out with no anesthetic except novocaine infiltration to the skin. Therefore only particularly tractable animals could be used, as the experiments necessitated keeping the animals strapped loosely on the table for several hours.

The animals were first prepared by making, under ether anesthesia, a burr hold in the skull about 1 cm. in diameter in the angle formed by the median bony ridge and the occipital ridge either on one or both sides. A cruciate incision was made in the dura mater and the soft tissues were closed. Two or more days were allowed to elapse before experiments were begun. With a little experience, it was then possible to introduce a blunt lumbar puncture needle into the slit-like lateral ventricle, allow 1 cc. of fluid to flow out and replace it with an equal volume of the drug to be used. The intraventricular injection experiments were controlled by others in which (1) pituitrin or pilocarpine was injected subcutaneously or

[1] Cushing, H., *Proc. Nat. Acad. Sciences*, 1931, **17**.

intramuscularly and (2) the dog was simply kept on the table, either with or without manipulations similar to the ventricular puncture. In only one of the dogs was all of these procedures done; in another the intraventricular injection of pituitrin was done 3 times at 2 day intervals.

Results. Records were kept usually at 15 minute intervals of rectal temperature, pulse, respiratory rate and character, panting, salivation, vomiting, diarrhea and changes in size of the pupils. It was hoped that the observation of the respiratory rate, and especially panting, might be found to be comparable to the sweating found in human beings as a mechanism for lowering body temperature. In this, however, we were disappointed, as panting frequently occurred even before the start of experiments and never seemed to have a definite relation to the injection of drugs. Changes in the pulse rate must also be interpreted cautiously because of the emotional element. The temperature was found to have variations independent of the experimental procedures. Hence the dogs were kept on the table for one or even 2 hours in order to be sure that the temperature had come to a constant level before injections were begun.

The positive results of the work may be summarized as follows:

Group I. Dogs at rest; no anesthesia; no drugs injected. 3 experiments. *Temperature.* Dropped 0.6° to 1.2°C. from the initial level in 2 dogs; rose 0.3° in the third dog; thereafter remained constant with variations of only about 0.1°. In one dog repeated unsuccessful attempts at ventricular puncture were followed by a transient rise of 0.4° followed immediately by a drop to 0.2° above the previous level. *Pulse.* Variations of 25 or more beats per minute either faster or slower but usually faster. *Respirations.* Fairly constant in rate, affected by occasional panting. No salivation, gastrointestinal disturbance or change in size of the pupils.

Group II. Subcutaneous or intramuscular pituitrin, 1 cc.; no anesthesia. 4 experiments. *Temperature.* In 2 dogs varied ±0.2°. In 2 dogs dropped 0.2° and 0.5°, beginning 2 hours and 1½ hours respectively after the injection. *Pulse.* In 3 experiments a drop in rate of 15 to 40 beats per minute lasting one or more hours. *Panting.* Occasional panting in 2 dogs; none in the other 2. *Salivation.* Very slight in one dog. Coryza in one dog.

Group III. Intraventricular Pituitrin, usually 1 cc., with anesthesia. Morphia and chloralose, 5 experiments; morphia and ether, one experiment. *Temperature.* In 2 dogs an immediate drop of about 0.2° within 15 to 30 minutes followed by a rise above the

initial level, in one dog of 1.1°, in one dog a drop of 1.4°, not beginning until 2 hours after the injection. In one case the temperature had not been allowed to become stabilized and fell 0.5° further, then rose 1°. In two dogs there was an immediate rise of 0.2° or 0.3°. *Salivation.* In 3 dogs was absent; in 3 was present but slight and occurred only 40 to 70 minutes after the injection. *Diarrhea.* None in 4 dogs; in 1 dog 2 explosive diarrheal movements occurred, 15 and 75 minutes respectively after the injection; in 1 dog an explosive diarrheal movement followed 3 minutes after additional pituitrin, 0.4 cc. was given intravenously.

Group IV. Intraventricular pituitrin usually 1 cc.; no anesthesia or sedative. 8 experiments. *Temperature.* Dropped 0.6° to 0.8° in 5 of 8 experiments (3 on the same dog); in 2 dogs a drop of 0.2° and 0.3° respectively; in one dog which was extremely restless, it rose 1.5°, then fell 0.8°. In practically all cases the drop began within 30 minutes. *Respirations.* Rate increase in all cases. *Panting.* In 7 experiments occurred within 10 minutes and in the 8th in 20 minutes, lasting from 1 to 7 hours. In 2 of these cases the dog had panted at intervals even before the injection. *Salivation.* In 5 cases occurred within 10 minutes; in remaining 3 appeared in 20, 25, and 60 minutes respectively; lasted from 1 to 3 hours. *Vomiting.* Retching in one dog, retching and vomiting in 1 dog. *Diarrhea.* In 3 cases more or less profuse, usually within about 30 minutes, lasting about an hour intermittently.

Group V. Subcutaneous and intramuscular pilocarpine (5 mg. in 1 cc. sterile water); no anesthesia. 3 experiments. *Temperature.* Rise of 0.5° to 1.1° in all 3 dogs starting 15 to 45 minutes after injection. *Pulse.* Rise in pulse rate of 40 to 80 beats per minute. *Panting.* None in 1 dog; moderate in 1 dog; present throughout experiment in 1 dog but more marked after injection. *Salivation.* Profuse, starting in 1 to 7 minutes and lasting 2 or more hours. *Vomiting.* Repeated vomiting in 1 dog, starting 4 minutes after injection and lasting about an hour. *Diarrhea.* Profuse in 2 dogs, starting in 30 minutes; none in third dog.

Group VI. Intraventricular pilocarpine (5 mg.), morphia and chloralose anesthesia. 2 experiments. The data of this group are insufficient for definite conclusions.

Group VII. Intraventricular pilocarpine, (4-5 mg.); no anesthesia. 4 experiments.* *Temperature.* Rise in temperature usually

* Since this work was finished, Light, Bishop and Kendall. (*J. Pharm. and Exp. Therap.*, 1932, **45**) in this laboratory have produced gastric lesions in rabbits by the intraventricular injection of small amounts of pilocarpine. In our experiments the gastric mucosa was not examined.

apparent within 30 minutes occurred in all 4 dogs, varying from 0.7° to 2.1°. Striking relation between temperature and respiration curves in that the temperature rose with a drop in respiratory rate. *Respirations and Panting.* Panting absent in 1 case; occasional during first 15 minutes in another; in remainder quite marked and seeming to inhibit the temperature rise. *Salivation.* Profuse in all cases, beginning in from 2 to 6 minutes and continuing for 2 to 5 hours. *Vomiting.* Occurred in one dog 5 minutes and again 8 minutes after injection; one dog vomited during attempt to tap ventricle. *Diarrhea.* In 1 dog 7 minutes after injection.

Discussion. In comparing the effect of intraventricular pituitrin and pilocarpine on man and dog 2 points must be kept in mind. First, because of differences in the 2 species, it is impossible to compare sweating and flushing, these being very difficult to recognize in the dog. Sweating of the foot pads was looked for but not found. Second, it is very difficult to keep a dog quiet on a table for several hours, and activity on the part of the dog obviously must influence body temperature, respirations, panting, etc. This is borne out by irregularity in results throughout all groups.

Salivation, vomiting and fall in body temperature after intraventricular pituitrin and pilocarpine without anesthesia were more or less consistently present except that pilocarpine caused a rise in temperature rather than a fall.† Our hope that the panting of dogs might be found to be comparable to the sweating of human beings was not realized, since panting was so easily evoked by various uncontrollable emotional stimuli. The pulse rate was likewise very labile depending on other factors also, thus interfering with the interpretation of injection effects.

The body temperature was found to be unstable. The room temperature was not carefully regulated or recorded, but the experiments were conducted in a room which was never uncomfortably warm or cold. It was almost the rule that the first temperature taken was a degree or so higher than the level which established itself when the dog had been on the table for some litle time. Unusual struggling or restlessness usually resulted in a rise in temperature as in one dog in Group IV in which we feel inclined to attribute the rise of 1.5° largely to this factor. This may also have contributed to the temperature rise following pilocarpine. The significance of the fall in temperature in the majority of instances

† Light in this laboratory placed 20 mg. of pilocarpine into the ventricles of 2 dogs with rise of temperature to 113 and 113.8°F. respectively with death in 45 minutes.

after intraventricular pituitrin in unanesthetized dogs is questionable. However, the rise after pilocarpine seems more definite. It is noteworthy that in the one dog in which the intraventricular injection of pituitrin was done 3 times, there was in each case a drop of 0.6° to 0.8°. In 2 dogs in which pituitrin was put into the cisterna magna there was likewise a fall of at least 0.5°.

Salivation was a constant effect of the intraventricular injection of either pituitrin or pilocarpine in unanesthetized animals. The effect was less rapid and less pronounced after pituitrin than after pilocarpine. The pilocarpine seemed to act no more quickly when given intramuscularly than when given intraventricularly or vice versa. In the anesthetized dogs pituitrin caused salivation in only 3 of 6 experiments, was only slight in amount and was slow to make its appearance. Subcutaneous pituitrin caused little or no salivation.

The gastro-intestinal effects were not pronounced. Retching or vomiting was only occasional. In 3 of 8 instances intraventricular pituitrin was followed by more or less active catharsis which is possibly of significance.

Cushing has shown that rectal avertin anesthesia inhibits the effect of pituitrin given into the ventricle. The inconstant and irregular results obtained here in a limited number of experiments under chloralose anesthesia although not conclusive suggest that the same is true for chloralose. While it is possible as we have shown to perform experiments such as here described without using any anesthesia whatever, the results are so easily influenced by many external factors that an anesthetic which will not block the centers in the neighborhood of the third ventricle, if such an anesthetic exists, is desirable if reliable results are to be obtained with dogs.

Summary. Pituitrin and pilocarpine have been administered to dogs by various routes and under various conditions. Intraventricular pituitrin in unanesthetized animals resulted in a fall in body temperature in the majority of cases, usually 0.6° or more, in distinct salivation, and in several instances in stimulation of the smooth muscle of the gastro-intestinal tract resulting in retching, vomiting or evacuation of the bowel. In animals anesthetized with chloralose there was less regularity in the effects produced, possibly because the anesthetic acts on the same centers as pituitrin.

Pilocarpine intraventricularly in unanesthetized dogs resulted in a rise in temperature and profuse salivation as did intramuscular pilocarpine. Gastro-intestinal effects occurred more frequently after intramuscular and subcutaneous pilocarpine than after intraventricular injection.

When these results are compared with those on man, it is evident that they are less conclusive and reveal marked differences in the reactions of man and dog to the effect of pituitrin and pilocarpine when introduced into the ventricles.

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Effect of Sympathomimetic and Parasympathomimetic Drugs on Motility of the Gastro-Intestinal Tract of Elasmobranch Fishes.

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It has been demonstrated (Nicholls[1]) that excised strips of the stomach of *Raja diaphanes* and *erinacea* suspended in a nutrient solution (NaCl, 16.38 gm.; urea, 21.6 gm.; KCl, 0.894 gm.; $CaCl_2$, 1.110 gm.; $NaHCO_3$, 0.378 gm.; NaH_2PO_4, 0.06 gm.; and dextrose, 1.0 gm. per litre of distilled water; with a pH of 7.8) will react to adrenalin, pilocarpin and acetyl-cholin.

Adrenalin (1:100,000 to 1:250,000) stimulates all parts of the stomach, raising the base-line rate and amplitude of the contractions. The only exception is in the antral region near the pyloric canal, where it stimulates in concentrations of 1:2,000,000, but inhibits in concentrations greater than 1:1,000,000. Adrenalin following pilocarpin or acetyl-cholin has an additive effect. Pilocarpin (1:250,000) and acetyl-cholin (1:100,000) stimulate all parts of the stomach. The former increases the rate and amplitude of the contractions, whereas the latter raises the base-line as well as increasing the rate and amplitude. Atropin (1:250,000) has no effect on the spontaneous contractions, but counteracts the effect of acetyl-cholin and pilocarpin, restoring the contractions to normal.

Thus it was shown that adrenalin not only stimulates isolated strips of the stomach (Dreyer[2] and Lutz[3]), but it has a definite inhibitory action, due probably to the hypersensitivity of a special region of the stomach to adrenalin.

The well marked effect of adrenalin, pilocarpin and acetyl-cholin lends further proof to the fact, first discovered by Bottazzi,[4] that

[1] Nicholls, J. V. V., *Contributions to Canadian Biology and Fisheries*, in press.

[2] Dreyer, N. B., *Trans. Nova Scotia Inst. Sci.*, 1928, **17**, 199.

[3] Lutz, B. R., *Biol. Bull.*, 1931, **61**, 93.

[4] Bottazzi, F., *Z. f. Biol.*, 1902, **43**, 372.