Physiology. — "*The Presence of Cardio-regulative Nerves in Petromyzon fluviatilis*". By J. B. ZWAARDEMAKER. (Communicated by Prof. H. ZWAARDEMAKER.)

(Communicated at the meeting of March 24, 1923).

In the 2nd edition of his "Physiologie des Kreislaufs" TIGERSTEDT [1]) remarks that inhibitory cardiac nerves are present in nearly all vertebrates. Only among the cyclostomata some exceptions are known. GREENE [2]) found that in Myxine electrical stimulation, starting from the brain, the spinal cord or the vagi did not affect the frequency of the heart-beat. CARLSON [3]) corroborated this finding and tried to extend the investigation to another group of cyclostomata, viz. the petromyzonta. At first he could work only on the larval form, in which cardio-regulative nerves appeared to be absent. Afterwards he examined adult animals [4]). When, in these experiments, he applied an electrical stimulus to the medulla oblongata on the level of the vagus nucleus, he noted a brief standstill, which was followed by an accelerated rhythm. From this he concludes that "the central nervous system is connected with the heart by ordinary augmentor and probably also by inhibitory nerves" (l. c. p. 231).

In the continuing volume of his "Vergleichende Anatomie der Myxinoiden" JOHANNES MÜLLER makes mention of a connection between. N. sympathicus and cardiac nerves [5]). He also adds some remarks about the N. vagus, for which I think it better to refer to the original work (l. c. p. 59 sqq.)

The first experiments which I made myself to ascertain whether in petromyzon fluviatilis any influence is exerted by the central nervous system upon the heart's action, yielded a negative result, which was in accordance with GREENE and with the first set of experiments performed by CARLSON [6]). However, I have been in a position to extend my research. In order to preclude

---

[1]) R. TIGERSTEDT, Die Physiologie des Kreislaufs II p. 319.

[2]) CH. W. GREENE, Amer. Journ. of Physiol. VI p. 318 1901.

[3]) A. J. CARLSON, Zeitschr. f. allg. Physiol. IV p. 259 1904.

[4]) A. J. CARLSON, Amer. Journ. of Physiol. XVI p. 230 1906.

[5]) J. MÜLLER, Fortsetzung der vergleichenden Anatomie der Myxinoiden p. 57. Berlin 1838.

[6]) J. B. ZWAARDEMAKER, Physiologendag Amsterdam Dec. 1922.

movements of the animal I curarized it beforehand. Paralysis of the skeletal muscles can, in fishes, be effected only with very large doses [1]). For my animals I used 4 mgr. tubo curari of which, 2 mgr., injected intraperitoneally, was sufficient to paralyze a 220 gr.-rat. after 7 minutes. This also plays an influence upon the vagus-function [2]), but this inconvenience could readily be obviated by the technique followed, because the synapses of the vagus are restored sooner than the motor innervation.

After the injection the animal was let alone until no "Stellreflexe" were distinguishable any longer. Also the gills are completely motionless then. At that juncture the cerebrum is severed from the rest of the nervous system by an incision posteriorly along the eyes. After this the cerebrum and the spinal cord are laid bare down to the second gill-hole. Now a straight glass cannula is inserted into the Vena cava dextra, through which the animal, in ventral position, is perfused during some time with RINGER's fluid, containing $6^1/_2$ gr. NaCl, 200 mgr. $NaHCO_3$, 200 mgr. $CaCl_2$, 200 mgr. KCl [3]). The surplus of curari is hereby gradually washed out. Through a window in the cartilagenous pericardium [4]) the atrium is fixed to a lever beneath the animal. Now two thin platinum electrodes are fixed, so as to be well visible, at the level where stimulation produces the effect aimed at. With strongly curarized animals it sometimes takes rather a long time before any effect can be distinguished. At that moment, however, the animal is perfectly quiet, and the experimenter can be sure that only the movements of the heart are registered. In subsequent periods of the perfusion also the contraction of the gills can be distinguished. The electrodes are connected with the secondary coil of an inductorium of DUBOIS-REYMOND, provided with a NEEF-hammer. An accumulator is connected up in the

---

[1]) *a.* J. SCHIFFER, Arch. f. Anat. u. Physiol. p. 453, 1868.

b. J. STEINER, ibid 1875.

c. BOLL, Mon. Ber. d. Kgl. Preuss. Akad. d. Wissensch. Nov. 1875.

d. J. STEINER, Das americanische Pfeilgift Curare p. 56.

c. and *d.* After R. BOEHM's article in Handbuch der experimentellen Pharmacologie II 1. Hälfte p. 183.

[2]) R. BOEHM, l. c. p. 202.

[3]) J. B. ZWAARDEMAKER, Diss. Utrecht 1922.

[4]) When the pericardium is being opened it all at once changes colour. Originally the heart is seen to loom vaguely through the transparent cartilaginous tissue with a bluish tint; after the opening the pericardium shows its own milkwhite colour, while the atrium now appears to lie at tbe bottom of the cavity. Apparently in the pericardium a negative pressure obtains, which of course is lost at the opening, so that the atrium partly collapses.

primary circuit. The Pfeilsignal, which was used sometimes (e.g. in the first figure), could not be placed in shunt, so it came in the primary circuit. The obtained coil-distances (C. d.) are smaller than when no signal is connected up. On stimulation we note a considerable acceleration shortly after the stimulus has been set up. If the stimulus continues a short time only (in fig. 1 5 seconds) the acceleration will be seen to disappear soon and to be substituted by a retardation ; in case the latter increases, the heart is brought to a standstill. After cessation of the negative chronotropic effect,

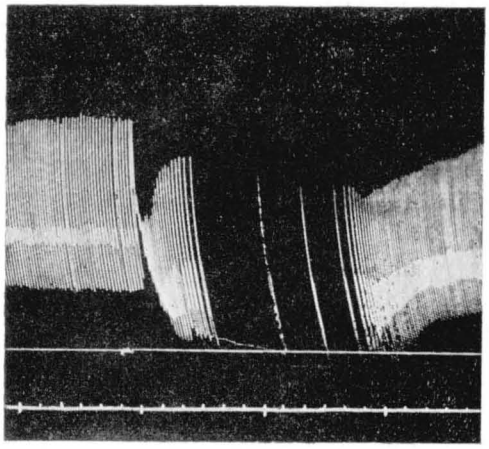

Fig. 1.
Accelerans-vagus effect.
Petromyzon fluviatilis. Perfused with RINGER's mixture. Stimulation for 5 seconds of medulla oblongata of the level of the exit of the N. vagus. C. d. 100.
The tracings from above downward: record of atrium movement
" " stimulus signal
" " time line 10 sec.

a new rhythm appears, more rapid than the original. A little later it gives way to the old rhythm. In fig. 1 the rhythm prior to the stimulation is ± 45 beats per minute, after the standstill the frequency amounts to 55. The action of side-currents upon the heartmuscle need not be taken into consideration in these experiments, because the effect appears only when a sharply defined area in the medulla oblongata is stimulated and the effect is destroyed again by a slight displacement of the electrodes. Besides this a great influence is exerted by summation. A stimulus, for instance, that produces no effect after 5 seconds, causes a distinct standstill after a longer period.

When instead of presenting a short stimulus, the current is sent through permanently, at first a marked quickening of the rhythm will be noted, attended with a marked positive inotropic effect. This is apparently an accelerans effect.

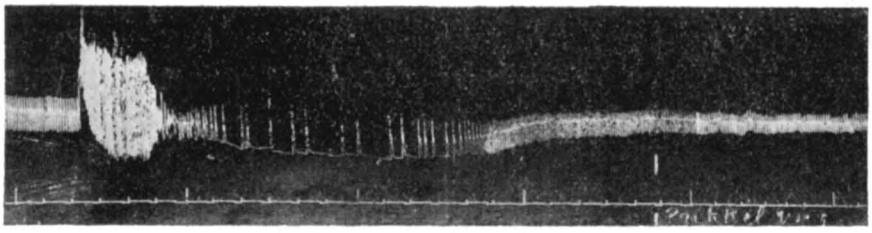

Fig. 2.

Petromyzon fluviatilis.

Fatigue of accelerans and vagus through permanent stimulation from medulla oblongata. The stimulus starts at the first elevations. C. d. 143. This continues as far as the stroke. Time 10 sec.

When breaking the current during this period a standstill will rapidly ensue, which will disappear again directly after fresh stimulation. When, however, the current passes continuously, a slower rhythm will appear after some time spontaneously (in fig. 2 ± 30 seconds), while at the same time the height of the contractions diminishes gradually. It is the transition to a distinct vagus-effect. When this rhythm has also continued for some time (in fig. 2 about 1 min.), it will change into a rhythm that is only slightly quicker than the normal, or does not differ from it at all, and will persist unaltered after the breaking of the current.

When perfusing the animal with a potassium-free uranium-containing, instead of a potassium-containing fluid we shall see that the phenomena are practically the same in the K-, and in the U-condition. First we see an acceleration, then a retardation, which in some cases is followed again by an acceleration. This, however, is never so pronounced as at the beginning of the stimulation.

What has been said above goes to show that:

1. in Petromyzon fluviatilis cardio-regulative nerves are present.
2. with the technique employed after the removal of curari the excitability of the cardiac nerves returns sooner than that of the motor nerves.
3. in the curarized animal the latent period of the accelerans is shorter than that of the vagus.

4. with long-continued stimulation the accelerans-effect is noticeable before the vagus-effect.

5. with brief stimulation the vagus effect appears only after cessation of the stimulation.

6. after cessation of the vagus-action an acceleration will sometimes follow, which is perhaps due to a longer after-effect of the accelerans-stimulation.
