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STUDY ON THE CENTRAL INFLUENCE OF SEROTONIN (5HYDROXYTRYPTAMINE) ON THE ARTERIAL BLOOD PRESSURE IN UNANAESTHETISED CATS

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Shortly after it's synthesis by *Hamlin* and *Fisher* (1951) 5hydroxytryptamine (5HT) has been identified by *Speetter and col.* (1952) and *Reid* (1952) with the serum vasoconstrictive factor described under different terms since the first observations by *Brodie* 1902. In 1948 *Rapport Green* and *Page* proposed the generally accepted name for this factor calling it serotonin which term has also been transfered to 5HT. In spite of the name serotonin had been found not only in the serum where it penetrates from blood platelets but also in the enterochromafian cells of gastric and intestinal mucosa (Enteramine of *Erspamer* and *Asero* 1952); then in spleen, liver, kidneys and lungs (*Weissbach, Waalkes, Udenfriend* 1957). In 1953 *Twarog* and *Page* claimed to find it also in the mammalian brain tissue. This was confirmed by *Amin, Crawford, Gaddum* (1954) and *Udenfriend, Weissbach, Bogdanski* (1957). The relatively large amount of 5HT within the brain seems to be particularly important especially considering numerable papers suggesting the endogenous 5HT to be the physiological neurohormon of central neurons in the brain stem and the cerebral cortex. (*Gaddum* 1953, *Wooley* and *Shaw* 1954, *Brodie* and *Shore* 1957, *Gluckman, Hart, Marazzi* 1957).

The precursour of serotonin 5hydroxytryptophan as well as serotonin antimetabolits (LSD 25, BOL) and agents enhancing it's acivity by blocking the monoamine oxidaze (Iproniazid) can be found on the principal place in pharmacology of psychotomimetic drugs nowadays. The majority of works concerning the circulatory effects of 5HT were performed in the period when it's central action was not sufficiently appreciated. Therefore the obtainable data about the 5HT central influence on the arterial blood pressure are still obscure and controversial. Moreover all previously described experiments were carried out on anaesthetised animals. That could not remain without influence on the results.

In 1953 *Page* and *Mac Cubbin* distinguished three components of the arterial blood pressure curve corresponding to three components of circulatory effect of 5HT. Their observations were also confirmed by *Comroe and coll.* (1953), *Schneider* and *Yonkman* 1954:

1. A rapid blood pressure fall connected with a marked slowing of the heart rate disappearing after bilateral vagotomy. This is supposed to be the *Van Bezold-Jarisch* phenomenon.
2. Arise in blood pressure of peripherial origin followed by.

3. A secondary prolonged decrease of the pressure due to the peripherial depression of vasomotor tone.

The interrelations of those components are greatly varied in different animals.

The spinal cord transection in bilaterally vagotomised cats and dogs usually results in considerable augmentation of the 5HT pressor effect (*Page* and *Mac Cubbin* 1953, *Schneider* and *Yonkman* 1954, *Freyburger and col.* 1954). Following *Page* and *Mac Cubbin* (1953) the hypotensive effect of 5HT occurs in vagotomised subjects due to the diminution of the neurogenic vasoconstrictor tone by means of the peripherial point of acting. This has been based upon the observation that serotonin caused a local vasodilatation when administered into the cróss-circulation of isoolated limb of dog A perfused by another dog B.

This phenomenon could not be seen after denervation of the isolated limb of dog A. The above mentioned findings have not been confirmed whatever by *Binet* and *Burstein* 1955 who worked following the proper method of isolated dog's limb autoperfused whith the aid of the Jouvelet's pump. In 1954 *Ginzel* and *Kottegoda* observed a slight decrease of blood pressure after administration of 5HT into the carotid or vertebral artery of cats in a deep chloralose anaesthesia after denrvation of carotid sinus. Atropine failed to suppress this effect. *Heymans* and *van den Houvel-Heymans* 1953 were able to demonstrate that the local application of 5HT to the wall of the carotid sinus caused stimulation of it's pressoreceptors resulting in a reflex fall of blood pressure. The purpose of our study was to distinguish whether the hypotensive action of 5HT in unanaethetised cats depends on the inhibition of the peripherial portion of the vasoconstrictor system as proposed by *Page* and *Mac Cubbin* or is due to the direct inhibitory influence of 5HT exerted upon the neural centers; and then which structury within the hypothalamus and brain stem are mostly influenced by 5HT.

METHODS

The experiments were carried out on 46 unanaesthetised cats operated in local 1% Novocaine analgesia. Immobilization of animals was obtained by the intravenous injection of 10 mg/kg of threeiodoethylen-three diethylylamin-ethoxy 1.3 benzenum („Flaxedil" Specia). This in contrast with other curare-like drugs remains without action on neural centers nor on the synaptic transmission in automatic ganglia (*Bonvallet Dell* 1954). In all subjects both vagi with aortic nerves were cut and the regions of the sinuses denervated bilaterally with 10% carbolic acid. This way the reflex phenomena could be avoided. Sections of the spinal cord were performed at the level of C_2 — C_3 after laminectomy of corresponding cervical vertebra. In some experiments the spinal cord was destroyed mechanically by the introduction of a probe into the vertebral canal. Sections through the hypothalamic region and brain stem were made with a 0,1 mm thin edge. The sections were performed in the stereotaxic apparatus of *Mac Culloch-Dell* following *Jaspers, Cosimo, Aimone Marsan* stereotaxic atlas. In every case after the experiment the brain was perfused with 10% formaline solution then removed and the levels of the sections were examined.

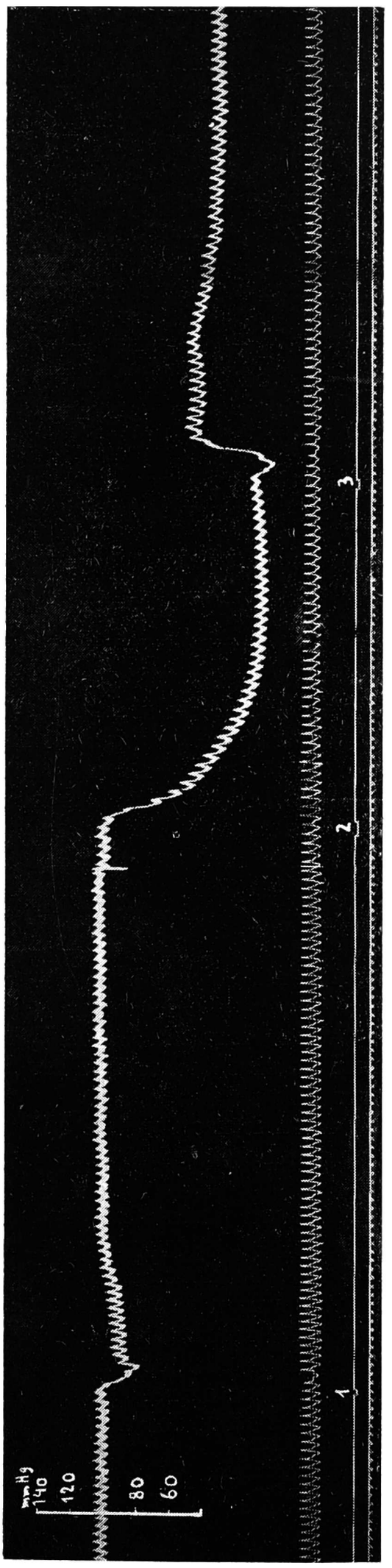


Fig. 1. Unanaesthetised cat immobilised by Flaxedil. Weight 2800 g both vagi cut, carotid sinus denervated. 1. — intravenous injection of 100 γ 5 HT, 2 — intravenous injection of 25 mg Pendiomide, 3 — repeated injection of 100 γ 5 HT. From top to bottom: arterial blood pressure in the right femoral artery, artificial respiration trace Deprez. signal, time 5″.

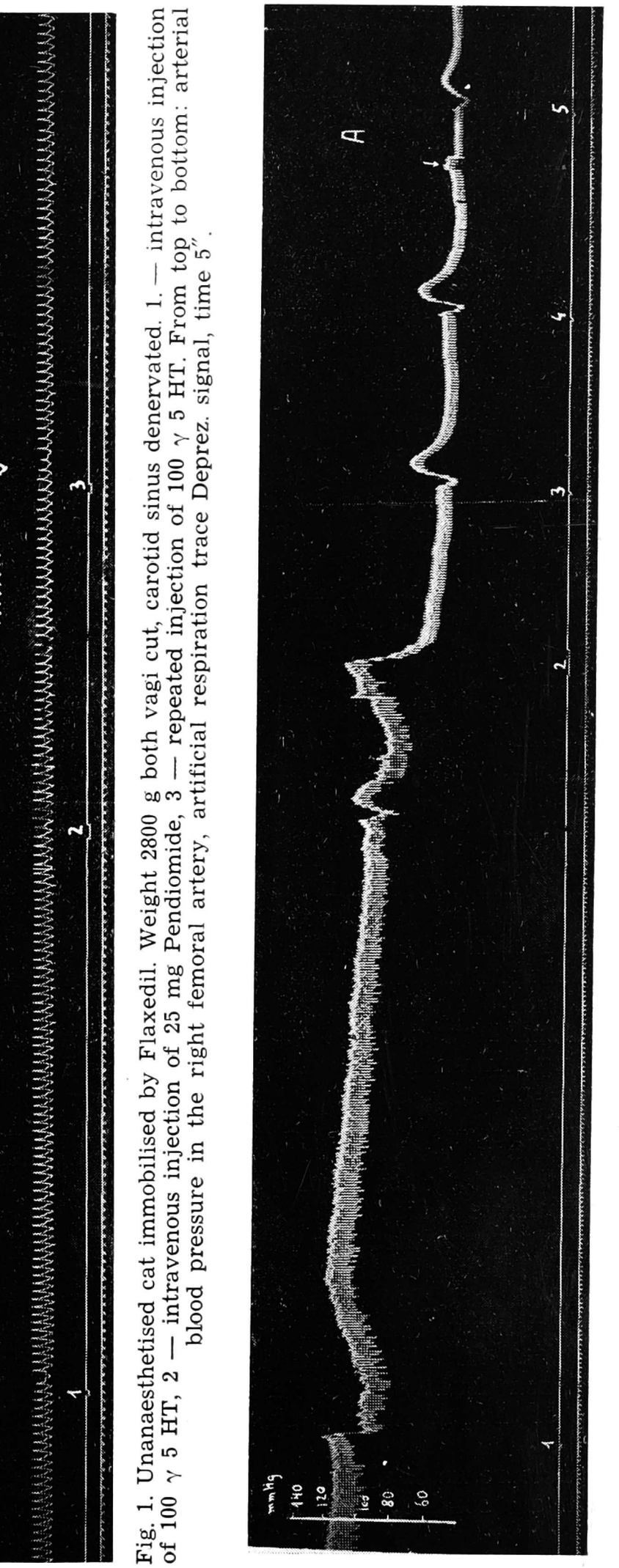


Fig. 2. Unanaesthetised cat immobilised by Flaxedil. Weight 2000 g. Both vagi cut, carotid sinus denervated. Artificial respiration, 1 — Intravenous injection 150 γ of 5 HT, 2 — Intravenous injection 4 mg of Decamethonium, 3, 4 — 150 γ of 5 HT intravenously. Between 4 and 5 bilateral adrenalectomy. 5 — Again 150 γ of 5 HT intravenously. From top to bottom: Arterial blood pressure in the right femoral artery, Deprez line, time maker 5″.

RESULTS

1. In cats with both vagi preserved serotonin caused a rapid and marked fall in blood pressure with bradycardia completing a typical cardio-pulmonary chemoreflex. After bilateral vagotomy and denervation of carotid sinuses three different types of reactions on 5HT were observed:

a) The hypotensive effect of a variable amplitude and duration.

b) The amphibaric effect with a short rise in blood pressure followed by a longpersistant depressive phase.

c) In some experiments a more or less pronounced hypertension was the only reaction to be observed.

The blocking of the synaptic ganglionic transmission with 5—10 mg/kg of Pendiomide or Decamethonium 3—5 mg/kg of body weight always caused a transformation of any of those reactions into a purely hypertensive one. (Fig. 1, 3). The stronger it was and longer the duration, the weaker were the hypotensive effects of 5HT before the administration of ganglioplegic drugs. The same result could be obtained by cutting the spinal cord just below the medulla. Following the entire destruction of the spinal cord an additional prolongation (10—20%) of 5HT pressory effect was noted (Fig. 2). Sometimes in spinal cats an abrupt drop of the pressure preceding the normal pressory reaction was observed. After *Reid* we suppose it to depend on the contraction of pulmonary veins induced by 5HT and followed by a secondary transient reduction of cardiac output.

In 1952 *Reid* showed evidence that serotonin possesses some stimulating properties towards the adrenal medulla. On the other hand a deprivation of the central nervous system is well known to augment the circulatory action of adrenalin. Therefore the question arose whether the increase of the hypertensive reaction to serotonin after blocking the synaptic transmission in sympathetic ganglia or after spinal destruction depended on the intermediary adrenalin mechanism. Nevertheless the experiments performed on bilaterally adrenalectomised spinal cats showed also the pressor action of serotonin. (Fig. 3).

The experiments described in the first series indicated that the hypotensive effect of 5HT observed in unanaesthetised cats depended on the nervous system. However the problem of the proper localization of it's point of acting remainded still obscure. This could be situated on the level of neural centers or conncted with the decrease of vasomotor tone on the periphery. Attempting to throw some light on this matter the same amounts of 5HT were alternately administered into the jugular vein and into the carotid artery brainwardly.

The hypotensive effect of serotonin administered into the brain circulation was much stronger then when given into the jugular vein. Moreover the fall in blood pressure following the application of 5HT into the carotid artery appeared also in these cases when the same dose of the drug given into the general circulation caused a marked rise in the pressure (Fig. 4). In the described experiments 5HT introduced into the brain circulation usually evoked the following three typs of reaction:

1) A drop of the pressure within 10—30 mm Hg persisting for about one minut.

2) A short decrease of the pressure immediately after the administration of 5HT with a secondary rise in the pressure.

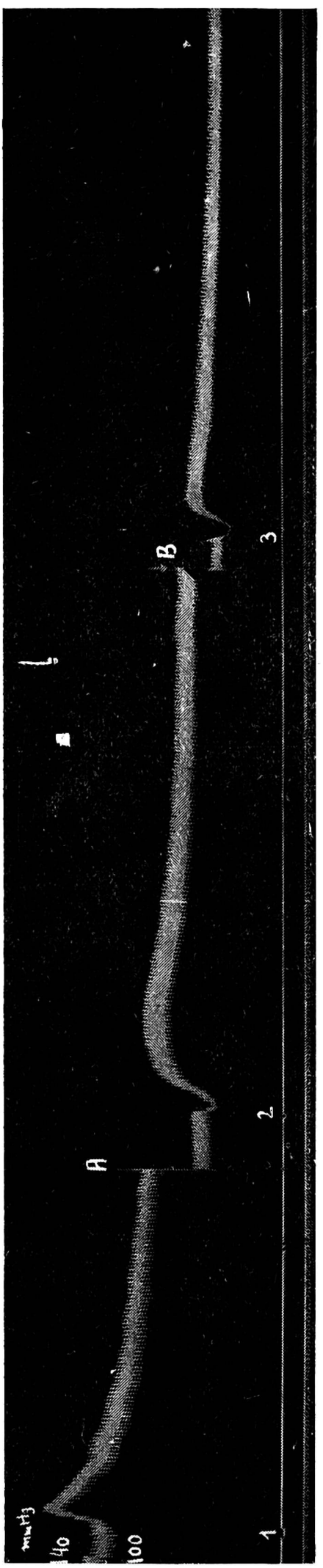
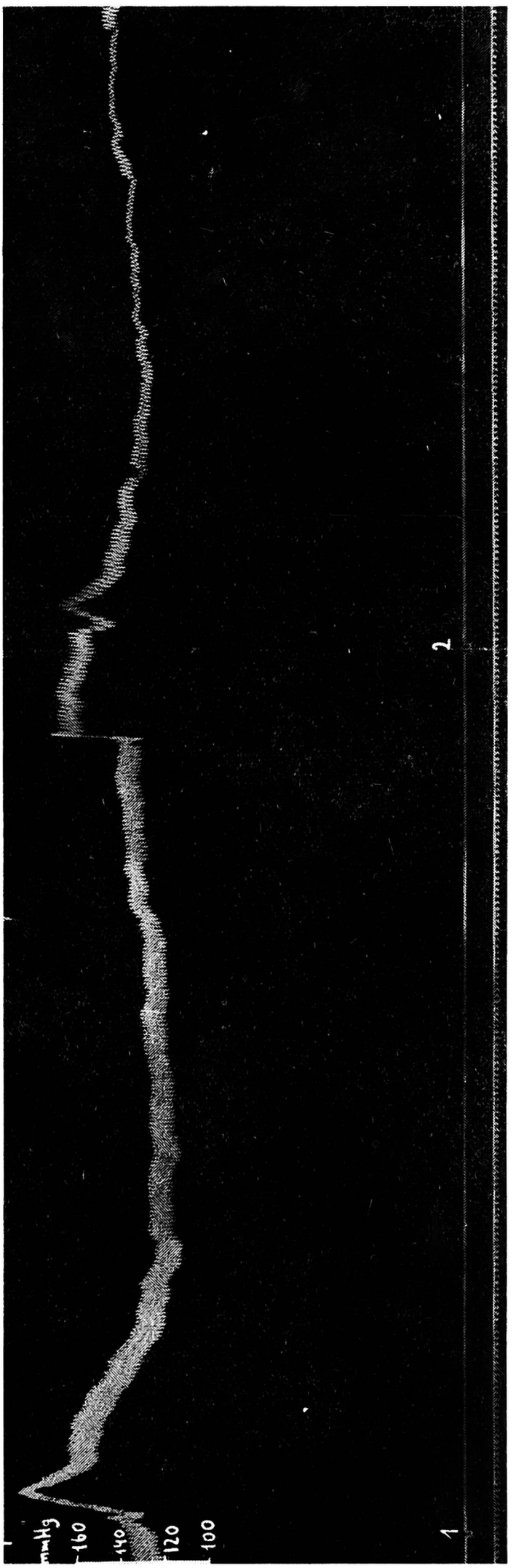


Fig. 3. Spinal cat 3000 g of weight Both vagi cut, 1 — Intravenous injection of 200 γ 5 HT, Arrow A — mechanical destruction of the spinal cord, 2 — 200 γ of 5 HT intravenously, Arrow B — bilateral adrenalectomy, 3 — 200 γ of 5 HT intravenously.



Flg. 4. Unanaesthetised cat, Flaxedil, weight 3000 g. Both vagi cut, carotid sinus denervated. 1 — Intravenous injection 150 γ of 5 HT, 2 — Injection 100 γ of 5 HT into the brain circulation trough the carotid artery. Tracings as on preceeding figures.

3) A slight depressive reaction of a rather long duration returning to normal after 10—15 minuts.

The section of the spinal cord as well as the use of Pendiomide or Decamothonium suppressed the hypotensive reaction previously observed. In these cases the only comprehensive effect was a rise in blood pressure appearing after the latent period that corresponded to the brain circulation time (Fig. 4).

When injecting 5HT into the carotid artery special care should be taken not to interrupt the blood flow through the artery. As it had been observed by *Holmes, Newman* and *Wolstenroft* (1958) a transient constriction of carofid arteries involved the ischemia of the auterior part of the brain leading to a partial decerebration. This by turns diminished the hipotensive reaction to 5HT.

II. In the second part of our study we investigated the arterial blood pressure of unanaesthetised cats after the intevenous administration of

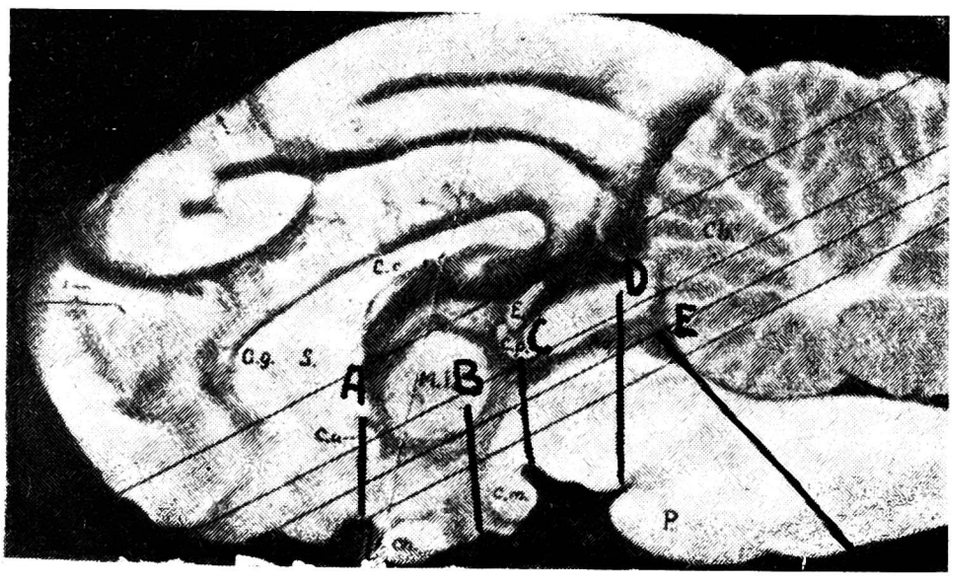


Fig. 5. The level of the sections through the brain stem.

5HT preceded by deprivation of different parts of the brain. On this purpose sections were made through the brain stem following the technic described by *Bonvallet, Dell* and *Hiebel* (1954). All cats were bilaterally vagotomised and both carotid sinuses were denervated. In order to prevent brain oedema we supplied 20—30 ml of 40% glucose solution intravenously. The enclosed photograph (Fig. 5) inlustrates the planes of the brain stem transections.

Section A (14—15 mm anteriorly from zero in the frontal plane), ran on the level of the anterior commisure separating the hypothalamus from the limbic system.

Section B (10—11 mm anteriorly from the frontal plane), ran on the level of the medial hypothalamus (ventromedial and dorsomedial nuclei) separating the paraventricular nucleus the supraoptic nucleus the preoptic area and the anterior hypothalamic area from the mammillary area. This way the anterior part of the hypothalamus containing mainly the parasympathetic centers (the trophotropic area of *Hess* 1948) was deprived from the remaining part of the brain.

Section C (5—6 mm from zero in the frontal plane backwardly

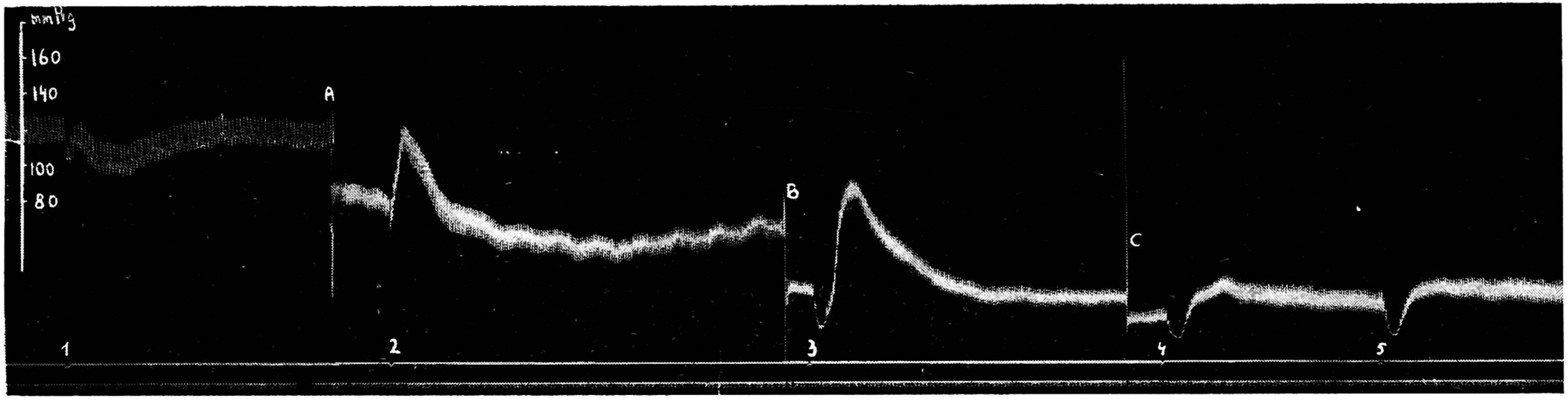


Fig. 6. Unanaesthetised and immobilised (Flaxedil) cat. Both vagi cut, carotid sinus denervated. 1 — Intravenous injection 100 γ of 5 HT, A — top of recording transection of the brain stem between the anterior and posterior colliculi. 2 — Intravenous injection 100 γ of 5 HT, B — transection of the spinal cord on the level of Tb 3. 3 — Intravenous injection 100 γ of 5 HT. C — Bilateral adrenalectomy, 4. 5 — Replated injections of 5 HT.

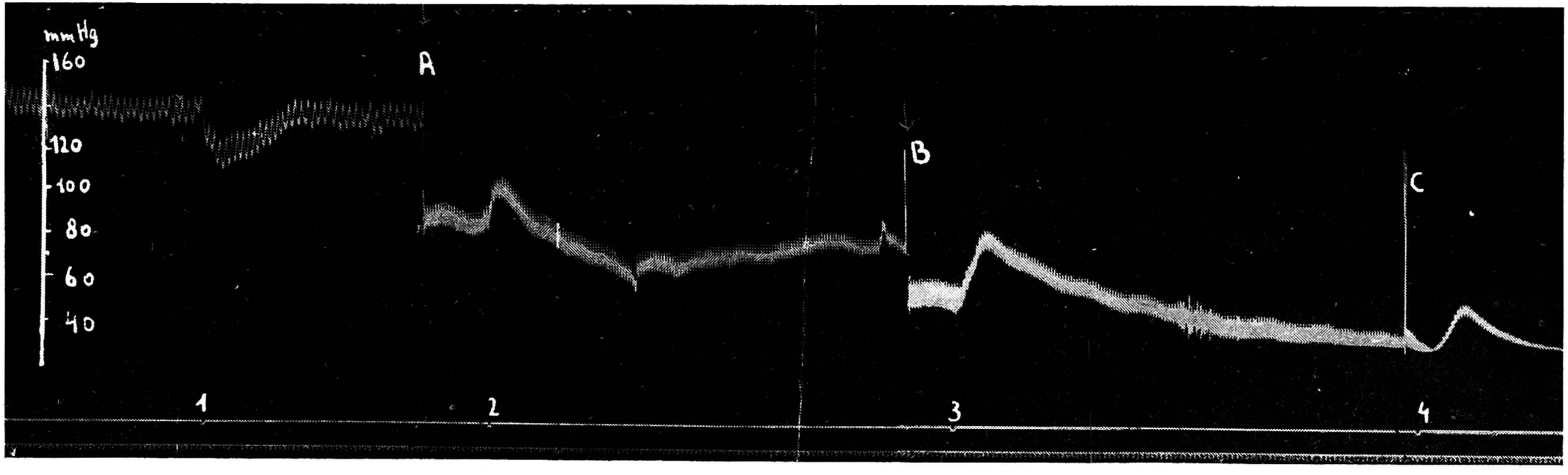


Fig. 6. Unanaesthetised and immobilised (Flaxedil) cat. Both vagi cut, carotid sinus denervated. 1 — Intravenous injection 100 γ of 5 HT, A — decerebrating transection through the brain stem on the level between the anterior and posterior colliculi. 2 — Intravenous injection of 100 γ of 5 HT, B — transection of the spinal cord. 3 — the same dose of 5 HT intravenously, C — mechanical destruction of the spinal cord. 4 — Intravenous injection of 100 γ of 5 HT.

from the mammillary bodies) separated the intire hypothalamus from the brain stem.

Section D ran between the anterior and posterior quadrigeminal colliculi corresponding to *Sherrington's* decerebrating transections.

Section E crossed the brain stem obliquely just behind the trapezoid body. Following *Bonvallet* and *Dell* (1954) this is the plain separating the activating part of the brain stem reticular formation from the inhibiting one in the epinephrine's action.

Section F was situated below the medulla.

Following section A the hypotensive effect of 5HT remained unchanged and sometimes was even more pronounced. The administration of 5HT

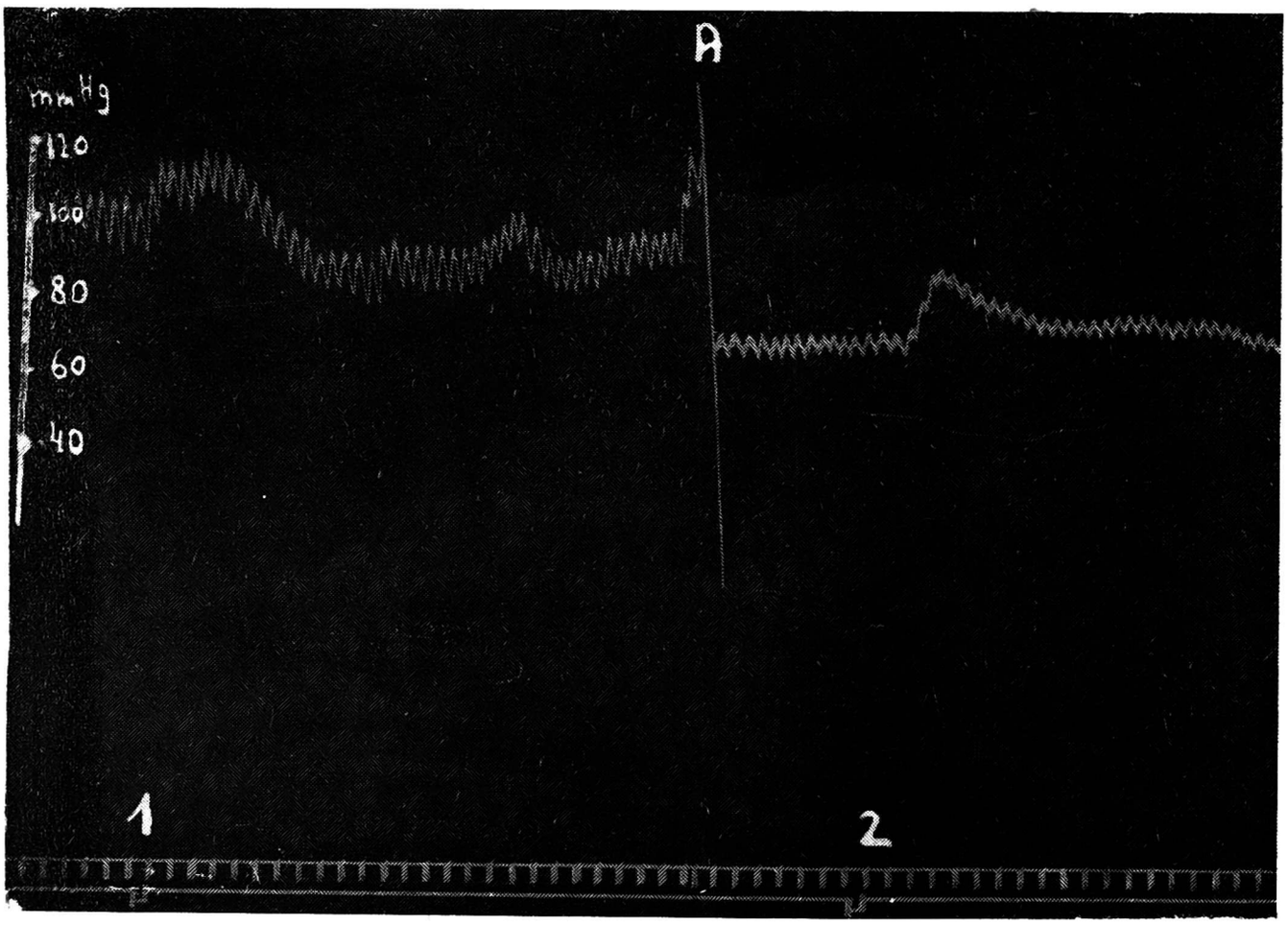


Fig. 8. Cat prepared as usual, 1 — Intravenous injection 100 γ of 5 HT A- the retrotrapezoid transection through the brain stem (see the text), 2 — Repeated intravenous injection 100 γ of 5 HT.

following section B evoked the appearance of a slight elevation preceding the fall in blood pressure. If in the control test the amphibaric reaction was observed section B markedly increased the pressory phase. Sections C and D also increased the hypertensive reaction to 5HT but did not abolish the secondary fall in the pressure. A complete suppression of the hypotensive effect wasn't obtained until the transection of the spinal cord below the medulla (section F) (Fig. 6 and 7). We noted almost the same result following section E (Fig. 8).

DISCUSSION

The results of our experiments indicate that the partial or complete deprivation of the central nervous system in unanaesthetised cats does not

prevent but on the contrary increases the hypertensive ceaction to 5HT; this also refers to adrenalectomised animals. Therefore the peripherial origin of hypertensive effect of 5HT is to be accepted. The augmentation of the pressory action of 5HT after the elimination of the central nervous system can be hardly atributed to the increased sensitivness of the circulatory system (denervation law). This seems to be obvious considering that the described phenomenon appears immediately after corresponding transections of the cerebro-spinal axis and after the destruction of the spinal cord. In bilaterally vagomised cats with denervated carotid sinuses the hypotensive action of 5HT depends upon the nervous system. The accentuation of this hypotensive reaction following the administration of 5HT into the brain circulation indicates the brain centers to be the point of it's action. This conclusion remains in contradiction to *Page's* and *Mac Cubbins's* one (1953). The opposite results of these authors could possibly be explained by the influence of narcosis and by the circumstance that their experiments were carried out on dogs.

The variability of the amphibaric circulatory reactions to 5HT introduced into an unanaesthetised animal submitted formerly to bilateral vagotomy and carotid denervation can be explained as interrelation between two opposite constituents:

a) The pressory effect exerted directly upon the circulatory system.

b) The hypotensive one due to neural centers.

Furthermore the hypotensive central action of 5HT extends itself upon different structures within the hypothalamus, the mesencepfalon, the pons and the medulla. This large area corresponds to the localisation of the brain stem reticular formation. The appearance of the peripherial pressory component following the deprivation of the anterior hypothalamus suggests it also to be influenced by 5HT.

It would be difficult however to agree with *Brodie* and *Shore* (1957) supposing that the central action of 5HT refers only to the parasympathetic neurons because atropine does not abolish the hypotensive effect after bilateral vagotomy. The central influence of 5HT upon the arterial blood pressure is probably due to the inhibition of vasomotor neurons within the descending reticular system. Phesumably this inhibition also involves to a certain degree the corresponding centers of the spinal cord; for it was observed that the additional complete destruction of the spinal cord prolonged in spinal animals the pressory effect of 5HT.

The hypotensive action of 5HT depends upon the regions of the brain containing normally the largest amount of 5HT. Presented after *Udenfriend, Weissbach* and *Bogdanski* (spectrophotophluorometric method) they are as follow:

The hypothalamus-	average	1.78 γ/g	of fresh tissue
The mesencephalon . . .		1.23 γ/g	„ „ „
The pons		0.33 γ/g	„ „ „
The medulla		0.55 γ/g	„ „ „

The distribution of 5Hydroxytryptophan decarboxylase which makes up serotonin (*Gaddum Giarman* 1956) and of monoaminoxydase metabolising it is much alike (*Bogdanski, Udenfriend* 1957). Therefore it may be supposed that the presence of 5HT within the brain stem is connected with the physiological function of the neurons responsible for vasodilata-

tion. *Hess* described some regions within the preoptic area of the anterior hypothalamus the stimulation of which caused a fall of blood pressure.

In 1952 *Eliasson, Lindgren* and *Uvnäs* found a dispersed neuron system situated in the anterior hypothalamus and in the brain stem related to an active vasodilatation by means of the cholinergic sympathetic fibers innervating the blood vessels of sceletal muscles. Stimulation of the centers described by *Eliasson, Lindgren* and *Uvnäs* leads to the dilatation of blood vessels in sceletal muscles without a comprehensive decrease in the general blood pressure. Moreover small doses of atropine suppresse the effect of stimulation of the centers connected with the cholinergic sympathetic fibers, it does not influence however the hypotensive reaction to 5HT.

Therefore the physiological role of 5HT seems to be rather connected with the system of neurons inhibiting vasomotor centers, then with the centers described by *Eliasson, Lindgren* and *Uvnäs*. The hypothesis as to the neurohormonal and mediatory role played by 5HT on the level of the brain stem centers requires further study. Doses of 5HT introduced into the organism in experimental conditions markedly exceeded the physiological amount of the substance within the brain stem. It has been known however that 5HT can hardly penetrate the blood-brain barrier. The percentage of 5HT reaching the brain tissue after an intravenous injectuin is difficult to establish. Further investigations would also be carried out upon the relationship between free 5HT and bound within the neurons.

Nevertheless some facts seem to confirm the proposed hypothesis: One of 5HT antimetabolits the Lisergic acid dietylamide (LSD 25) penetrating freely through the blood-brain barrier produces the elevation of the arterial blood pressure (*Wooley, Shaw* 1957). On the other hand another potent antiserotonin 1benzyl-2,5dimetylserotonin(BAS) to which the blood-brain barrier is unpermeable does not effect the brain and fails to produce the elevation of the blood pressure. On the contrary the substance is capable of reducing the high blood pressure of human patients suffering from essential hypertension (*Wooley, Shaw* 1957).

The above mentioned facts agree with the hypothesis that 5HT is a mediator of the neurons inhibiting the neurogenic vasomotor tone.

CONCLUSIONS

1. In unanaesthetised cats with both vagi transected and carotid sinus denervated the intravenous injection of 30—50 γ kg of body weight of 5 HT produces the amphibaric respons.

2. Following the administration of ganglioplegic drugs such as Pendiomid and Decamethonium as well as after decapitation a rise in blood pressure is the only reaction to 5HT. Destruction of the spinal cord elongates the pressory effects observed in spinal cats. The additional bilateral adrenalectomy does not suppress the pressory reaction in such cats.

3. In comparison with the same dose of the drug administered into the general circulation 5HT injected into the brain circulation produces a more pronounced hypotensive reaction. The transection of the spinal cord as well as the ganglioplegic abolishes this reaction.

4. The ischemia obtained by the constriction of both carotid arteries increases the pressory reaction to 5HT.

5. The transection of the brain stem at the level of the anterior commisure does not abolish the hypotensive reaction to 5HT. The section running through the middle part of the hypothalamus increases the pressory effect of 5HT but does not abolish the secondary fall in the pressure. Section crossing the brain stem between the anterior and the posterior colliculi as well as the retrotrapezoid one gradually increases the pressory reaction and diminishes the secondary fall in the pressure.

6. The proposed hypothesis is that 5HT is a mediator of the reticular neurons responsible for the central inhibition of vasomotor tone.

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ИССЛЕДОВАНИЕ ЦЕНТРАЛЬНОГО ВЛИЯНИЯ СЕРОТОНИНА НА КРОВЯНОЕ ДАВЛЕНИЕ НЕНАРКОТИРИЗОВАННЫХ КОШЕК