Does the Amount of Epinephrine in the Peripheral Blood after Morphine Exceed the Limits Determinable by the Frog Legs Vessels Method?

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The epinephrine no doubt exists in the general blood, when it is abundantly poured out from the suprarenal glands, either by asphyxiation, or bleeding, or cooling, or hypoglycemia, etc., all of excessive degree. The intravenous introduction of the adrenaline solution with almost the same velocity, as the epinephrine is charged from the suprarenal into the general blood circulation, brings about an elevation of the general blood pressure, blood sugar concentration, etc., in proportion with the degree of abnormal conditions.

So far we are short of sufficiently sensitive method of estimating epinephrine both biologically and chemically.

Some years ago, a new solution for the rabbit ear vessels method, was devised by Schlossmann.¹⁾ It should enable us to determine the amount of epinephrine the blood with certainty and such a sensibility as 1:10,¹² but, it was soon found rather harmful to our disappointment.²⁾ Then, by modifying this solution Z. Kanowoka³⁾ devised another solution for determining the epinephrine in blood, but it does not increase sensibility of this method at all.

I

A few years later Gyoku reported that he was able to prove in rabbits that the epinephrine increases in the ear vein blood on subcutaneous application of morphine, and this increasing tendency reaches its acme 2 hours after the injection. Laewen-Trendelenburg's frog leg perfusion

The publication of the papers from my Laboratory has, to my great regret, been considerably postponed, owing chiefly to my being absent from the Laboratory work for past three years in order to devote myself to the duties connected with the University administration.

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came into application.

Morphine is also one of the most potent agents to discharge the epinephrine from the suprarenals; the maximum output rate is 0.0006 mgrm per kilo per minute from one side gland in the normal dog;⁵⁾ the drug was applied either hypodermically or intravenously.

Expecting to realize how Gyoku alleged to find epinephrine in the general blood of rabbits on applying this potent drug, I attempted some experiments, the results being given below, although he simply used the fluid containing Na-citrate and made no mention of the sensibility in particular.

Methods: Rabbits of both sexes were experimented on. Morphine hydrochloride was injected under the skin as a 2 per cent solution in the physiological saline fluid, in the dose of 50 mg per kilo of body weight. The vein blood was taken from the denervated ear. The arterial blood was collected from the left carotid through a small cannula, about 1 cc blood being discarded at first. **The Frog Legs Vessels Method:** Ringer containing 0.2% sodium citrate was used, and the initial number of drops flowing out from the vein cannula was adjusted to about 30 per minute, and the blood specimen or the adrenaline chloride solution, made from that of Sankyo Co., as the control was introduced into the arterial cannula. The blood specimen was prepared as follows. 0.5 cc of the blood was taken into a syringe, in which 0.5 cc of 4% Na-citrate solution was drawn up beforehand, so the blood was diluted 2 times. Very seldom a further dilution was made. 1 cc of such a specimen was introduced into the arterial cannula of the preparation of 10 seconds. The introduction of the blood specimen was always performed within 2 seconds after taking out the blood. **The Rabbit Intestine Segment Method:** The arterial blood was poured into a glass cylinder, and then it was placed in an ice-chest to be defibrinated. Then the epinephrine was estimated by means of the rabbit intestine segment, in the usual manner of this Laboratory. The indifferent blood was taken from another not morphinized rabbit or dog.

Results:

On receiving the morphine rabbits became quiet gradually, with the respiration deep and tardy. In most cases the heart rate became slow and sometimes irregular, and it was so during the whole course of the experiment. The ear vein blood became dark half an hour after morphine what lasted longer than 2 hours.

Blood samples were taken before the injection, and thereafter as well, at intervals of one, two or three hours.

The blood from 9 normal rabbits was applied to the test by the rabbit intestine segment method and the frog legs vessels method as well. The

former method disclosed that the inhibitory power of 0.5 cc of the defibrinated arterial blood samples taken before and after the morphine administration was less than 0.000 025 mgrm. adrenaline chloride, i.e. $1:2 \times 10^7$. Namely no epinephrine was thus observable even after the morphine injection.

The latter method gave apparently some different features, which seemed to duplicate the findings of Gyoku; that is, 1 cc the citrate vein blood, taken before the injection caused a remarkable decrease in the number of drops flowed out from the preparation, and that taken out after the morphine acted stronger. Generally only one specimen was tested after the injection; it seems to me, however, not highly probable that the specimen taken two hours after the administration acts most strongly vasoconstrictively. Figs. 1 & 2 show, for instance, a reverse relation clearly.

In evaluating the results obtained by means of the vessel perfusion, a special consideration shall be given so that the blood specimen might be injected into the preparation within a constant but possibly short interval each time, for example 20 seconds (10 seconds from taking out the blood to the start of injection; then 10 seconds to its end.), or else the vasoconstrictor power will develop rapidly, which may in time affect the estimation seriously. As a consequence, the time when specimens should be applied is definitely fixed; neither the order of application nor the time when they are to be applied should be altered by way of trial. In this respect the experimental conditions are highly restricted in this method of determination, only the application of the control experiment with the adrenaline solution of a known concentration, which roughly corresponds to the specimen to be matched the vasoconstrictive force, can lessen the difficulties inherent to this method.

Fig. 3 may be given to show the general course of the matter. 0.000 05 mgrm. adrenaline chloride was introduced, which was soon followed by the specimen before the administration. About two and a half hours later, that is about two hours after the morphine, the specimen was taken out and tested. Soon later 0.000 05 mgrm. adrenaline chloride was applied. The initial drop number was 32, 30, 32 and 25 a minute, but on taking them uniformly as 30, Fig. 3 was constructed.

It is then not unfair to take the vasoconstrictor power of the ear vein blood before and after as the same, on taking the effect of one and the same amount of adrenaline chloride as the control. Other five experiments showed quite the same nature of results.

Of the carotid blood, and the defibrinated carotid blood similar experiments were carried on three rabbits with the wholly similar outcome; only it may be said that the vasoconstrictive power of the defibrated blood is

was taken.

Fig. 2: Normal rabbit; 3 hrs after the morphine the blood was taken.

Fig. 3: Normal rabbit; 2 hrs after m., Adr. (1:1000) 0.05 cc.

Fig. 4: Doubly splanchnicotomized rabbit; 2 hrs after m., Adr. 0.1.

Fig. 5: Doubly decapsulated rabbit; 1 hr (triangles), 2 hrs after m., Adr. 0.05 2 hrs after m.

originally far stronger than the others.

II

Similar experiments as above were carried on further on the rabbits either doubly splanchnicotomized or doubly suprarenalectomized. The neurotomy was done 16 days, 2 days, 17 days and 9 months previously, and the last removal of capsule was done 21–53 days on four rabbits before the morphine experiments.

These sets of experiments were carried out to see whether or not we are able to duplicate the findings of Hayama, that the carotid blood of the rabbits, deprived of the splanchnic nerves or of the suprarenal bodies bilaterally, does not possess any trace of the vasoconstrictor power (rabbit ear perfusion method), but this power was found in the same amount in those operated on in the above mentioned manner, as in the normal individuals. One example from each group of animal will be presented in Fig. 4 & 5. No explanations are needed.

Carotid blood was taken from two suprarenalectomized rabbits, besides the ear vein blood specimens. The arterial blood was tested either immediately or after defibrination; the results did not differ from those obtained in normal individuals.

SUMMARY.

Contrary to some writers, the present author has never come to see the existence of epinephrine in the general blood of rabbits, poisoned by the morphine. Both the rabbit intestine segment method and the frog legs vessels method were employed. Exactly said that the epinephrine does not exist in the peripheral blood beyond the limits determinable by means of both the methods.

The failure of the previous writers might be explained probably in this way that they did not pay any attention upon the fluctuation of sensibility of the vessel perfusion method against epinephrine or adrenaline in the course of experiment. The second writer carried on his experiment on the animal while it was near moribund, or very weakly.

References.

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