

STUDIES ON THE BLOOD VESSELS IN THE MEMBRANES OF CHICK EMBRYOS

PART II. REACTIONS OF THE BLOOD VESSELS IN THE VASCULAR MEMBRANES

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In recent years there has been lively discussion on the behavior of the vascular system especially concerning the dependence of reactions of the vessels on the nervous system (1, 2, 3, 4). In order to advance knowledge of this matter and to make clear what actually takes place, and to attempt to indicate the significance of these processes, it has become necessary to enlarge this study by examining a new and a greater variety of material. The blood vessels of the vascular membranes of chick embryos seem especially adapted to the ends in view. Membranes containing the vessels can be easily examined both microscopically and macroscopically after removing the shell and the shell membrane. Stimuli of any desirable kind may then be easily applied. Since it has been shown (Part I) that these tissues contain no nerves, the course of the reaction takes on a special interest.

A. The Effect of Electrical Stimuli of Varying Strength on the Blood Vessels

Faradic Stimulation.—The technique used was the same as that employed in Part I. The electrode consisted of two copper wires each a millimeter thick and separated a distance of 1 mm. by means of collodion. The points of the electrodes, which alone came in contact with the tissue, were polished together with the collodion in which they were embedded, by means of sandpaper. Eggs were used after having been incubated 4 days.

In order to facilitate comparison, stimuli were made in the following experiments always at the same place, namely, the final pre-

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capillary branches of the artery which passes upwards towards the head of the embryo and to the right.

Experiment 1.—A very weak faradic current just strong enough to create a sensation of prickling on the moistened lips was used. After the electrodes had been placed, without exerting pressure, upon the appropriate vessels, current was allowed to flow through the electrodes for 10 seconds. They were then removed for 10 seconds in order to observe the effect which had been created, and then were replaced for a further 10 seconds. The duration of the stimulation was measured with a stop-watch. After the stimulus had been applied for 20 seconds, dilatation of the vessels appeared. The flow of blood in them continued to be rapid. In the surrounding vessels there was no change. We designate this stage in which there is dilatation with rapid flow "the stage of fluxion," adopting Ricker's term. 1 minute after the end of stimulation, the original diameter and rate of flow had returned.

From this experiment we made the following inference: The weakest possible faradic current brings about a brief stage of fluxion.

Experiment 2.—In this case a faradic current of medium strength which causes merely a burning sense when applied to the lips was used. The site of the stimulus was the same as in Experiment 1. The duration of the stimulus was 20 seconds, during the course of which there was an interruption of 10 seconds. At the end of 20 seconds, contraction of the blood vessels developed. The rate of flow was rapid. If the stimulus was continued for 20 seconds longer, the blood vessels shut. In the capillaries just peripheral, the flow ceased, so that the blood in them was motionless. Others were empty. The larger arteries central to these appeared unchanged. The flow of blood just proximal to the point of contraction was rhythmic. 5 minutes after the end of the stimulus the original diameter of the vessel was reestablished and the rate of flow was as before.

We made the following inference: A faradic current of medium strength brings about contraction of that arterial branch which has been stimulated.

Experiment 3.—A faradic current which was just strong enough still to be borne when applied to the lips, and which caused an unpleasant sensation of burning was used in the same way and at the same site as in Experiments 1 and 2. After 20 seconds there appeared a spindle-shaped dilatation, the degree of dilatation being greater than in Experiment 1. The rate of flow was at first slow, and then stopped after a number of seconds at a point further out than the site stimulated. Central to this point the dilated vessel was filled with blood. The blood corpuscles agglutinated so that they were no longer individually identifiable. Adopting Ricker's term we designate this "the stage of stasis." Capillaries peripheral to the site of

stimulus were empty. Just central and just peripheral to that portion of the affected vessels which was dilated, but within the region of influence of the stimulus, the arterial branch was contracted; in consequence, the dilated portion appeared as distinctly spindle-shaped. The spindle-shaped dilatation and the contraction of that part of the vessel just central to it appeared to occur synchronously. The capillaries beyond, that is to say peripheral to the vessels so affected, were empty. Transition between the dark red region of stasis and that region beyond which appeared light owing to the absence of blood could be recognized macroscopically as a sharp line. Stasis persisted throughout the observation and could not be influenced by any new stimulus.

From these findings we have drawn the inference that a strong faradic current brings about stasis.

In order to ascertain whether and to what extent dilatation depends on the pressure of the inflowing blood stream, we carried out the following experiment.

We placed a pincette which shut off the flow of blood on a small artery. A stimulus of the same strength as that employed in Experiment 3 was then applied to the artery. Dilatation of the same degree took place. The blood in the dilated vessel was, however, a lighter red since it was distributed in a greater space. After 2 minutes the pincette was removed. The dilated vessel was thereupon filled with blood and the spindle appeared to be quite full.

There appears, therefore, to have taken place, genuine primary dilatation with filling as a secondary phenomenon. The dilatation may become so great and the spindle so filled with blood that in young specimens the total volume of blood contained in the embryos has been seen to collect in the dilated area. Under these circumstances the heart continued to beat but appeared as a mere shadow, seeing that it was empty of blood.

Under similar experimental conditions a weak faradic current brings about dilatation of the arterioles of the vascular membrane with increased rate of flow, the stage of fluxion, a current of medium strength, contraction to the point of closure, and a strong current stasis of the blood in a dilated vessel. It appears therefore that the reaction depends upon the strength of the current. It is necessary next to ascertain whether other stimuli have the same consequences.

B. *The Effect of Various Other Stimuli*

(a) *Mechanical Stimuli*.—In this case the stimulus consisted of touching the locus with a blunt glass rod. The site of the stimulus was as before. Because of the greater size of the glass rod, small arteries as well as the capillaries were involved.

The *gentlest possible* contact was established 3 times in 1 second, the intervals between being naturally brief. As a result the small arteries and capillaries dilated, becoming larger than those in the neighborhood. The rate of flow in the dilated vessels was rapid. This is the stage of fluxion. *Stimuli of medium strength* were applied 3 times, at the same rate as before and to the same site but with somewhat greater pressure. The small arteries and capillaries now contracted and disappeared. The site which was stimulated appeared as a lighter spot, empty of blood. The vessels in the surrounding region were not visibly altered and exhibited a rapid flow. 5 minutes later the small arteries again filled from the side of the heart and 1 minute later still the general appearance was the same as before the application of the stimulus. *Strong stimuli* consisted in bringing the glass rod in contact with the vessels 3 times, using quick strong strokes. An enormous dilatation of the small arteries and capillaries took place at once in the region stimulated. Centrally and peripherally, waist-like contractions were observed. The capillaries which were related anatomically to the area, but which were outside the region affected by the stimulus, were empty. An hour afterwards no change had taken place, stasis still persisting.

We drew the following inference: Depending upon the strength of the stimulus, mechanical irritation brings about a stage either of fluxion or one of contraction or one of stasis.

(b) *Ammonia*.—The site of stimulation was as before. The stimulating substance was applied through the needle of a small syringe.

Experiment 1.—When 0.5 cc. of a 1 per cent solution was injected, dilatation took place at once as well as a rapid flow of blood at the site of stimulation.

Experiment 2.—10 minutes after the stage of fluxion had been brought about as in the first experiment, the injection of ammonia (0.5 cc.) was repeated. The capillaries and the smallest arteries contracted. When a third injection of the same amount was made there was moderate stasis in the capillaries whereas the small arteries were contracted.

Experiment 3.—When 0.1 cc. of a 5 per cent solution of ammonia in Ringer's solution was injected slowly, the arterial branch which was affected dilated enormously at once while complete cessation of blood flow through it took place. Dilatation was so complete that stasis did not develop as in the previous experiment but instead coagulation of the blood. Agglutinated cells floated about in a clear medium within the vessels.

We have drawn the following inference: Ammonia brings about the stage of fluxion, of contraction, or of stasis and hemorrhage, according to the concentration used and the period during which it acts. Strong concentrations cause coagulation (pseudostasis).

We have chosen the experiments described as examples of the effects of 3 qualitatively different kinds of stimuli. But we have also made experiments with mustard oil, with silver nitrate, with potassium iodide and with oil of camphor. In principle the action of all was the same. It was possible by grading the concentration and the dose, to bring about the same effects as when the faradic current, the glass rod or ammonia was employed. Since the stimuli used were able to produce an effect on blood vessels in tissue in which there were no nervous elements, we have been forced to conclude that they have their effect upon the cells themselves and that these have an inherent power to exhibit the motions which we have observed.

The action of adrenalin as reported in the first of these papers was inconstant. Atropin had no action at all even in concentrations as high as 1 per cent. Cold and heat had no distinct effect. For that reason we were unable to utilize different degrees of temperature as means of establishing grades of reaction on the part of the vessels.

In our experiments we have found then that with effective stimuli there is a systematic relation between the strength of the stimulus and the reaction of the blood vessels, in the sense described by Ricker in the case of blood vessels in which a nervous apparatus exists. A weak current as we have shown is followed by the stage of fluxion, one of medium strength by that of contraction and a strong one by stasis. The general rule of the relatedness of a particular form of reaction to a particular intensity of stimulus is exhibited also by the non-innervated blood vessels studied in these experiments and by the kinds of stimuli which were employed. Our observations therefore confirm Ricker's theory concerning the laws governing the relation between reactions and the strength of stimuli. In the case of blood vessels which are not innervated it appears that the strength of the stimuli not the nature of the stimulus constitutes the determining factor. The same forms of reaction were found whenever it was possible to grade the strength of the stimuli irrespective of their kind.

C. The Effect of a Given Stimulus upon Different Portions of the Vascular System

In the experiments so far reported, stimuli were always permitted to exert their effect upon vessels of a like diameter. We have next to report our study of the effects of the same stimulus on capillaries, small arteries and on arterial main stems. For this purpose we have employed faradic current, mechanical stimulus, or one of the chemicals already mentioned, mustard oil, silver nitrate, potassium iodide or the oil of camphor.

Experiment 1.—We stimulated a capillary area with a very weak current for 20 seconds. There resulted the stage of fluxion. When the same stimulus was applied to small arteries or the main stems of arteries, no visible reaction took place and we drew the following inference: A stimulus which brings about the stage of fluxion in capillaries has no effect upon arteries.

In *Experiment 2* a stimulus of medium strength applied for 20 seconds to a small artery caused contraction. A stimulus of the same strength applied for the same length of time to the capillaries caused stasis. If the same stimulus was applied for the same length of time to an arterial stem, no distinct effect ensued although the appearance of a slight degree of dilatation occurred occasionally. From this experiment we draw the inference that a stimulus which causes contraction in small arteries causes stasis in the capillaries.

Experiment 3.—If a strong stimulus was applied for 20 seconds to an arterial stem, stasis took place in the vessel which had undergone spindle-shaped dilatation. The same stimulus if applied to small arteries or capillaries likewise caused stasis. And we draw the inference that a stimulus which causes stasis in arterial stems does so likewise in small arteries and capillaries.

SUMMARY

A comparison of the effect of stimulation upon the arterial subdivisions shows that these differ in irritability. The capillaries are most irritable, then come the small arterial branches, and last the main stems of arteries, these being the least irritable. The non-innervated blood vessels of the vascular membranes exhibit therefore the behavior described by G. Ricker in the case of those which have a nervous apparatus. We have demonstrated accordingly similar properties in vessels which are not innervated as in those which are. Certain differences exist however. The action of adrenalin on the vessels of the vascular membrane is inconstant and certain other sub-

stances are wholly ineffective. Ricker's experiments have advanced knowledge on the regularity of the vascular reactions and have paved the way for these experiments of ours. The result of our experiments with vessels free of nerves suggests that a reexamination may be fruitful of the mechanism which obtains in innervated vessels. There can be no doubt that nerves play a rôle in the behavior of vessels; it appears now to be necessary to define more accurately precisely what this is.

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