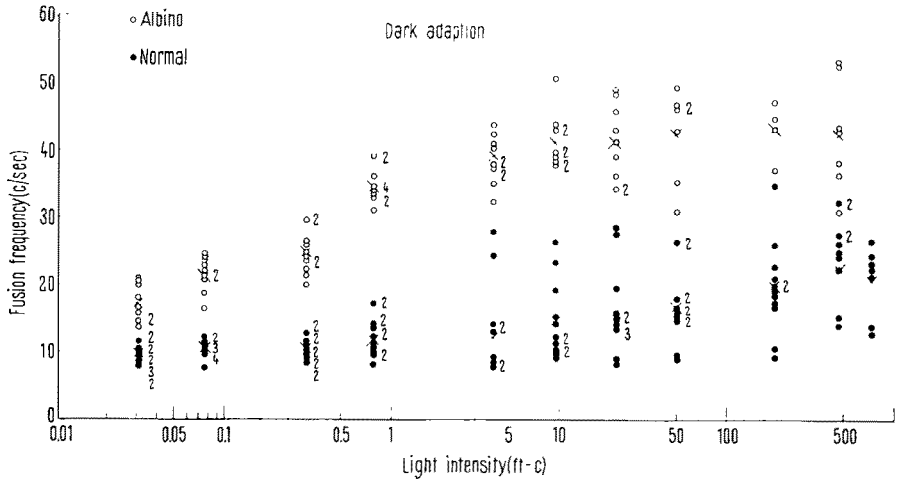

Fig. 2. ERG-FFF values for 6 each of dark-adapted albino and normal trout. Only the left eyes were used and 2 experiments were made at each intensity. The crosses indicate the means.

used (Figure 2). The MFF is attained at 10 ft-c in the case of the albino while it is attained at about 460 ft-c in the normal trout's case. The shift from rods to cones occurs at intensities higher than 0.3 ft-c in the albino while it does so at intensities higher than 4.0 ft-c in the case of the normal trout (Figure 2).

It is evident from these results that the lack of REP in the albino is primarily, if not wholly responsible for the differences observed (Figures 1 and 2). Due to the absence of the pigment, not only a greater amount of light enters the eye, but this light impinges on the visual cells twice, as a result of its reflexion by the sclera. In the normal trout the light will be absorbed by the REP and not reflected. In the light-adapted state the rods of the normal fish are shielded by the REP while in the dark-adapted state at least the cone outer segments are masked by the REP. This will also reduce the amount of response. In the albino, both in the light- and dark-adapted states, the rods as well as the cones will be responding and to almost twice the quantity of light. The cones, of course, will respond only to intensities which exceed their threshold which may be safely assumed to be between 0.1 and 1.0 ft-c on the basis of earlier investigations with related salmonids[2,3,16].

Résumé. Les Fréquences de Fusion de l'Electrorétinogramme (FFF) ont été enregistrées chez les truites mouchetées (*Salvelinus fontinalis*) normales et albinos. Chez les poissons adaptés à la lumière ainsi qu'à l'obscurité les valeurs sont plus élevées chez les albinos que chez les animaux normaux. Les valeurs maximum de FFF sont aussi atteintes à des intensités moins élevées chez les albinos. Ces différences sont attribuables à l'entrée d'une plus grande quantité de lumière ainsi qu'à sa réflection par la sclérotique, ce qui est due au manque de pigment épithelial rétinien.

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An Improved Bioassay Method for Kinins

Recently, naturally occurring vasoactive polypeptides, kinins, have been supposed by many researchers to play important roles under pathological conditions such as acute pancreatitis[1], carcinoid syndrome[2], various allergic diseases[3,4], or inflammations[5]. However, there are few reports giving direct evidence that kinins are involved in such diseases.

The following problems make it difficult to study the kinins: (1) they exist in extremely small amounts in plasma and in tissue; (2) they are rapidly destroyed by kininase which is present simultaneously in the blood or in the fluid.

In 1963, Binia et al.[6] used a dog's hind-quarter as an organ preparation for the bioassay of plasma kinins. However, this method was unsuitable, since the preparation was not sensitive and produced no vasodilatation with amounts of kinin of less than 10 ng. In the present study, a 20 times more sensitive method for the assay of kinin is presented utilizing the hind-quarter of a rabbit.

Albino rabbits of both sexes weighing 3.0–4.0 kg were used. The animals were anaesthetized by an i.v. injection of urethane (1.0 g/kg body weight). The carotid artery was exposed and a polyethylene cannula was inserted. One femoral artery was then isolated and cannulated

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with another polyethylene cannula. A thick rubber tube and a T-form polyethylene tube were tightly fitted to the end of the femoral cannula. The carotid artery was connected to the femoral artery with a vinyl tube containing physiological saline, and an extracorporeal circuit was established (Figure 1). In addition to this procedure, the jugular vein was also cannulated with polyethylene tube. The supplements of heparin and urethane, if needed, were injected through this cannula.

Perfusion of the hind-quarter of the rabbit was performed at a constant flow rate by a Sigma motor pump interposed between the carotid and the femoral artery. By adjusting this pump, the flow rates could be controlled at our disposal. A perfusion pressure was set at 100–120 mm Hg by changing the perfusing blood volume, and was continuously recorded by means of an electronic manometer (Nihon Kohden, MP-4). The air cushion was used to remove the pulsation of the motor. Anticoagulation was achieved by heparin (500 U/kg body weight).

The entire extracorporeal circuit was composed of polyethylene and vinyl tubes. Glassware was not used, because the possibility of kinin formation by glass-activated kallikrein should be excluded from the assay system.

Samples and standard bradykinin were injected into the rubber tube set into the circuit as close as possible to the femoral artery. The activity of kinin in the sample was measured by comparing its depressor effect with that of synthetic bradykinin. The usual injection volume was 0.1 ml, but it could be increased to 0.2 ml if necessary. The injections were made slowly, taking about 10 sec, in order to avoid the volume effect on the perfusion pressure record.

As illustrated in Figure 2, dose-dependent vasodilatation, as recorded by the fall in the pressure, could be clearly demonstrated in this preparation. Figure 3 shows the relationship between the doses of kinin and their depressor responses in 5 rabbits. The linear relationship was recognized in the dose of 0.5–3.0 ng of synthetic bradykinin. However, practically no further increase of effect is seen at doses higher than 3.0 ng. In all animals, 0.5 ng of bradykinin produced a detectable vasodilatation.

Furthermore, the other vasoactive substances, adenosine triphosphate (ATP), acetylcholine, histamine, serotonin and prostaglandin E_1, were studied with regard to their actions on this organ. As illustrated in Figure 4, histamine and serotonin produced pressor responses in the doses of 1000 times greater than that of synthetic bradykinin. On the other hand, depressor responses were observed by the intraarterial injection of ATP, acetylcholine and prostaglandin E_1. Prostaglandin E_1 produced a long-lasting vasodilatation. In the cases of acetylcholine and ATP, however, a similar vasodepressor effect to that of synthetic bradykinin was observed. Accordingly, contamination of these substances should be completely avoided

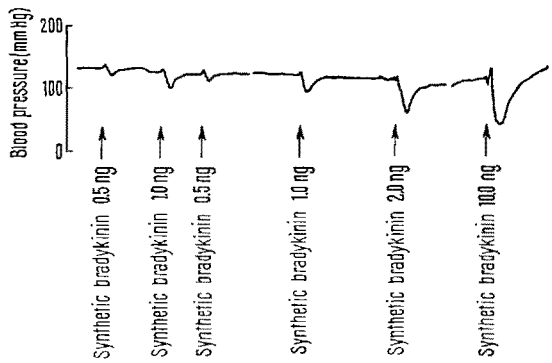

Fig. 2. Dose-response curves of synthetic bradykinin on the rabbit hind-quarter preparation.

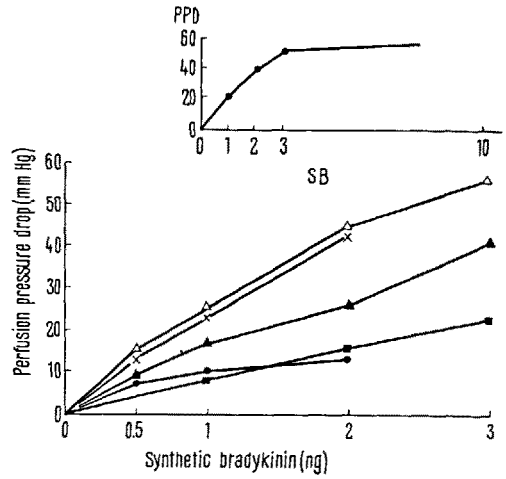

Fig. 3. The relationship between the doses of synthetic bradykinin and the perfusion pressure drop of the hind-quarter preparation in 5 rabbits. PPD, perfusion pressure drop (mm Hg); SB, synthetic bradykinin (ng).

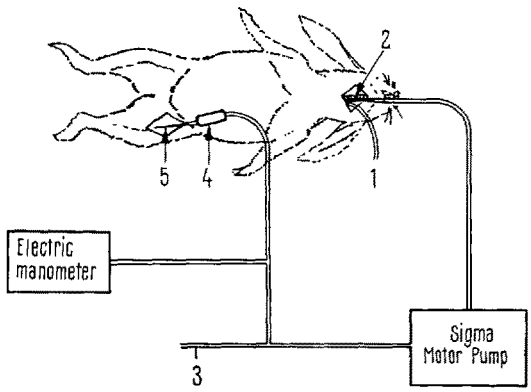

Fig. 1. The autoperfused hind-quarter preparation of a rabbit. (1) Left jugular vein; (2) left common carotid artery; (3) air cushion; (4) rubber tube; (5) left femoral artery.

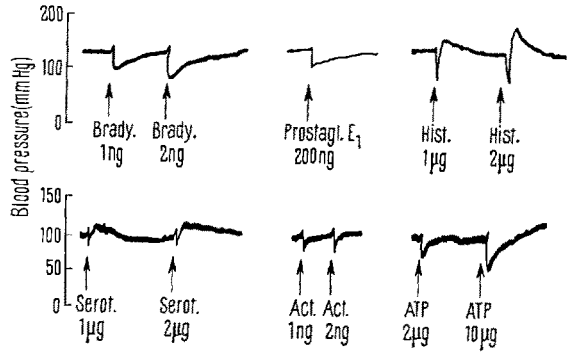

Fig. 4. The vasoactive effects of bradykinin (Brady.), prostaglandin E_1 (Prostagl. E_1), histamine (Hist.), serotonin (Serot.), acetylcholine (act.) and adenosine triphosphate (ATP) on the rabbit hind-quarter preparations.

or, if this is not possible, the action of them should be blocked by specific inhibitors when the activity of kinin is determined by the autoperfused hind-quarter of the rabbit.

During the past 10 years, biological assay methods were applied to the estimation of kinins. An isolated rat uterus and a guinea-pig ileum were widely used as the biological preparations[7]. On isolated organs, however, spontaneous contraction and relaxation are frequently observed. These movements can seldom be suppressed completely by atropine, antihistaminics, changing the concentrations of Ca in the perfusing solution, or changing the temperature of the bath fluid. Accordingly, it was difficult to obtain accurate results in these preparations.

On the contrary, the constant response to kinin was found on the above-mentioned rabbit hind-quarter preparation, because the fluctuation of the base line of the perfusion pressure was minimal under suitable anaesthetic conditions. Rabbit femoral artery was very sensitive to kinin and responded with sharp dose-dependent vasodilatation. This preparation was applicable for the assay of amounts of kinin less than 3 ng. To test the accuracy of this method, test samples of synthetic bradykinin were prepared by another investigator and estimated without knowledge of its concentrations. Only 5% experimental error was recorded. From these results, we concluded that the autoperfused hind-quarter of rabbit was an excellent organ for the estimation of a small quantity of kinin.

The only disadvantage of this method is that ATP and acetylcholine produce a similar depressor action to that of kinin. Accordingly, a contamination of these substances should be kept in mind when the activity of kinin in a crude sample is measured. However, we have developed a new extraction method for measuring the plasma kinin activity[8]. With this method, more than 95% of acetylcholine is eliminated and ATP completely removed.

Therefore, the combination of our extraction procedure with the bioassay using the autoperfused hind-quarter preparation of the rabbit seems to be very suitable for determining the activity of plasma kinin at the present stage[9,10].

Zusammenfassung. Unter Ausnützung der besonderen Empfindlichkeit der Femoralarterie des Kaninchens für Kinin wurde die autoperfusionierte Hinterextremität beobachtet. Intraarterielle Injektion von 0,5 ng synthetisiertem Bradykinin ergab eine messbare, dosisabhängige Vasodilatation in fast allen Versuchstieren. Dieses Kreislaufpräparat erweist sich zur Kinin-Bestimmung als besonders geeignet.

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[10] Acknowledgments: The authors are grateful to Prof. T. Torikai for his leadership, and Dr. H. Ono and Mr. K. Sato of the Department of Internal Medicine, Tohoku University School of Medicine, Sendai, for their technical assistance in this study. Synthetic bradykinin was kindly supplied by the Sandoz Pharmaceutical Co., and prostaglandin E_1 by Dr. J. E. Pike of the Upjohn Co.
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Magnesium-Calcium Antagonism in the Contraction of Arterioles

Resistance vessels dilate when exposed to a high concentration of Mg in blood. The vasodilatation could be the consequence of diminished output of transmitter substance from tonically active vasoconstrictor nerve endings, or of a direct action of the Mg on the smooth muscle of arterioles. Observations reported in the literature are compatible with both interpretations[1,2].

To study further the mode of action of Mg on resistance vessels of the peripheral circulation, experiments were done on 9 anaesthetized, atropinized dogs. The right gracilis muscle was perfused with the animals arterial blood[3] using a sigma-motor pump to provide a constant flow rate. At the beginning of the experiment the blood flow was adjusted to produce a normal pressure head (90–110 mmHg). Changes of resistance were registered as changes of inflow pressure. Flow rate was monitored by a drop counter at the venous outflow, and determined from time to time by collecting blood during a measured time interval. The systemic arterial pressure was also recorded. $MgCl_2$, $CaCl_2$ and other drugs were delivered into the tubing of the perfusion pump by motor driven-syringes. Recirculation of injected Mg and Ca was not permitted. Mg and Ca concentrations were determined[4] in the plasma collected from the outflow cannula. Packed cell volume (PCV) of blood samples was also measured. The PCV was slowly decreasing throughout the experiments. The right abdominal sympathetic trunk was stimulated with shielded electrodes between L3 and L4 segments with 10 msec supramaximal pulses. The central connections of the sympathetic were severed.

In the control state stimulation of the abdominal sympathetic trunk produced powerful vasoconstrictor responses in the vessels of the perfused gracilis muscle. When $MgCl_2$ was added to the blood, the inflow pressure dropped and stimulation of the vasoconstrictor fibres became less effective. At the same time the pressor response evoked by injected noradrenaline or vasopressine also diminished (Figure 1). Mixing $CaCl_2$ with $MgCl_2$ restored the pressor effect of sympathetic stimulation and of constrictor drugs (Figures 1 and 2).

Under the influence of excess Mg the vasoconstriction caused by injected noradrenaline was in most cases at least as much depressed as the vasoconstriction caused by sympathetic stimulation. For example in the experiment

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