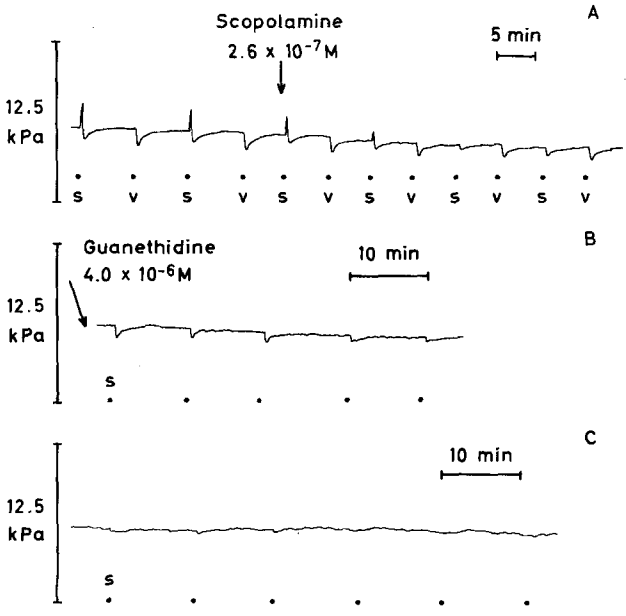

Fig. 2. Intraluminal pressure registration of isolated guinea-pig trachea. *A* Stellate ganglion stimulation (s), by comparison, alternated with vagus nerve stimulation (v) (parameters 25 Hz, 30 V, 1 msec). In this preparation, vagus nerve stimulation resulted in relaxation only. Scopolamine abolishes the excitatory sympathetic response. In *B* and *C*, which are continuous records, the sympathetic inhibitory response is blocked by guanethidine in a low concentration.

levels between the vagal trunk and the sympathetic chain are well documented[7,10,12]. Our denervation experiments suggest that the adrenergic fibres which reach the trachea via the cervical vagus nerve derive from anastomoses close to the origin of the vagus nerve.

The nonadrenergic, noncholinergic inhibitory nerves[4,5] of the trachea seem to have a preganglionic vagal supply. This would be in accordance with findings on lungs from amphibians and reptiles, and be an analogy to the nonadrenergic, noncholinergic inhibitory innervation of the stomach[13]. In conclusion, the guinea-pig trachea is one more example demonstrating the complexity of the autonomic innervation of different organs.

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Laser Doppler microscope in an oblique-backward mode and pulsatile blood flow velocity in pulmonary arteriole

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Summary. Blood flow velocity, pulsatile in correspondence to cardiac events, in pulmonary arterioles of anesthetized bullfrogs could be measured on lung surfaces covered with a water-containing plastic disc by means of a laser Doppler microscope arranged in an oblique-backward mode.

Laser Doppler microscope[3,4] was originally designed in a see-through position, i.e. in a dual-beams forward-scatter mode, in which the laser tube and a combination of the microscope and photomultiplier were set in a face-to-face position and the tissue to be studied was placed between them. The Doppler shift of laser beams scattered in the probing area of a micro-vessel was detected by the photomultiplier situating in the opposite side of the laser source. Therefore, the laser Doppler microscope was hitherto used for measuring blood flow velocity in a thin transparent tissue of frog web[5,6]. If it is used in a reflection position, i.e. in an oblique backwared-scatter mode, where the microscope-photomultiplier is placed abreast of the laser tube so as to detect the laser light scattered in the probing area at an acute angle with the direction of the incident laser beams, the applicability of the laser Doppler microscope may be much increased, because it permits the observation of micro-vessel existing just beneath the surface of the organs. However, the well-known difficulty occurring in the optical system having such a mode is the shot noise caused by the light reflected on the tissue surface. In this paper, we study a method of minimizing the disturbance by the shot noise and of quantitatively measuring blood flow velocity in a pulmonary arteriole.

Experimental method. The experimental arrangement is schematically shown in figure 1. An unilateral lung of the anesthetized Bullfrog was exposed by thoracotomy and inflated by means of an intratracheal catheter. The incident dual beams were crossed in an arteriole of the alveolus of the exposed lung. The microscope-photomultiplier was focussed to the probing area of the arteriole on the alveolus. An area of the lung surface under observation was covered with a water-containing plastic disc, Soft 38® (thickness, 0.23 mm, curvature, 8.8 and diameter, 13 mm, Nichicon, Nagoya, Japan), to study the effects of covering

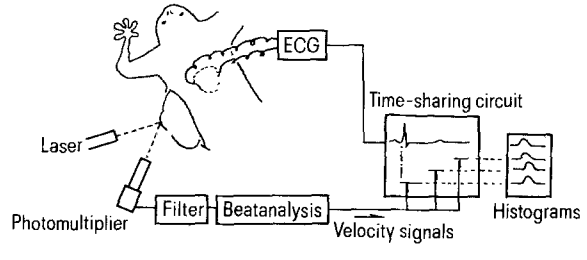

Fig. 1. Schematic illustration of an experimental arrangement.

the surface. The high frequency noise and the pedestal component involved in the output signals from the photomultiplier were filtered off by a dual cascade filter consisting of high-pass (cutoff frequency 0.5 kHz) and low-pass filters (cutoff frequency, 4.5 kHz). The frequency of output signals was measured as a reciprocal of the wave period by means of the beatsignal analyzer designed as previously described[3] (manufactured by Nihonkagakukogyo Co. Japan). The time-sharing circuit[7] was triggered by an ECG signal which was obtained from the 2 needle electrodes placed near the heart and the time-shared signals were separately stored in 16-channels of frequency appearance histograms, corresponding sequentially to the lapse of time after the occurrence of R-wave on ECG. Each of 16 channels covered the time period of 60 msec but summarized the signals obtained during the first 30 msec (i.e. the sampling time was half the covering time interval). The time-shared histograms of electric signals were displayed on a X-Y plotter by means of a computer system.

Results and discussion. An example of the time-shared histograms is shown in figure 2, A, where the abscissa and ordinate represent the wave period of signals obtained from the arteriole of the exposed lung wall without any covering, and the appearance frequency in an arbitrary unit, respectively. The lowest histogram shows flow velocity appearance frequency occurring from 0 to 30 msec after R-wave. The 2nd histogram shows the same relation occurring during the time period of 60–90 msec after R-wave, and the other histograms follow the same relation occurring in the time lapse of every 30 msec. The peak frequency of 16 histograms scarcely shows any change in response to the cardiac events (the difference among the peak frequencies is nearly 8% of the mean value).

When the lung surface was covered with a suitable plastic disc containing 30% water, the histograms of figure 2, B were formed in the range of lower frequency than in figure 2, A, and their peak frequencies show clear changes in response to the cardiac cycle. Namely the histograms begin to deviate toward the higher region at 120 msec (mean flow velocity 3.2 mm/sec) after R-wave, attain the maximum value at 360 msec (mean flow velocity 3.7 mm/sec), and then gradually approach the initial level. The largest difference among the peak frequencies is nearly 25% of the mean value, which clearly exceeds the random deviation occurring in the histograms obtained without any covering. The very careful examination of the present Doppler microscope including the optical and signal-analyzing systems shows that there is no cause in the microscope system producing errors in beat signals, and that any changes appearing between A and B of figure 2 are due to the flow object itself with and without covering.

When the low-pass filter in the case of 'with covering' was switched from the usually employed cutoff frequency 4.5 kHz to 2 kHz, the histograms could not be obtained any more. This suggests that the histograms summarized only the Doppler shift frequencies characteristic of the blood flow velocity. Meanwhile, it was observed in the case of 'without any covering' that clear histograms were formed at any frequency of low-pass filter. This phenomenon can be explained by the contribution of shot noise which contains a relatively wide range of frequency. Furthermore, the effect of the covering on the observed changes was studied in an in vitro test. Water suspending 5 μM polystylol microspheres was forced to flow through a glass capillary. The flow velocity of the suspension was measured with and without covering by means of the laser Doppler microscope, while the flow rate of the suspension was simply measured by means of a messcylinder. Within 5% error, the flow velocity obtained with covering agreed with that predicted by the simply measured flow rate. On the other hand, the measurement without any covering yielded values far different from the predicted values.

A photograph of the visual field shown in figure 3 indicates how the dual incident beams proceed and are reflected at the interface when the lung surface was covered with the disc. The spots A and A′ are the reflections at the interface between plastic surface and air. The reflection here is strong but outside of the probing area, because the photomultiplier is equipped with a mask having a small pinhole, by which only the light coming from an extremely small

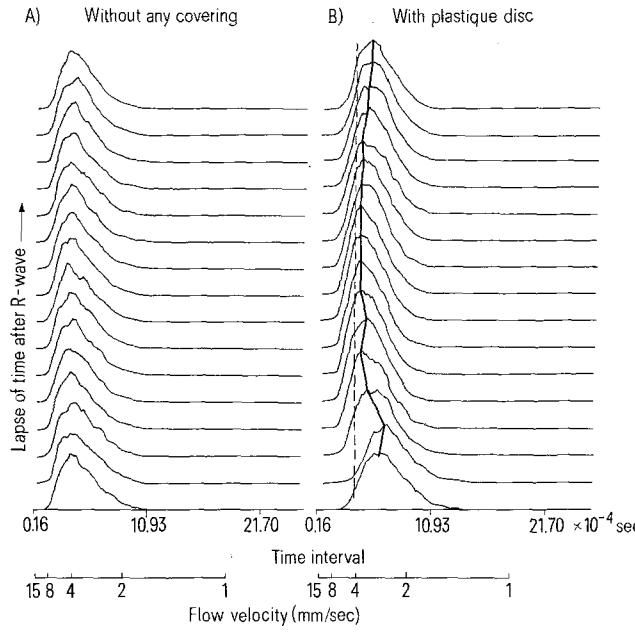

Fig. 2. Time-shared 16-histograms (A), obtained in a large arteriole having the diameter of 60 μm of the exposed lung surface without any covering, which show no essential changes in accordance with the cardiac events, and those (B), obtained in the disc-covered lung, deviating with cardiac events. Peak velocities in histogram (B) are connected with the straight line, while the vertical broken line indicates the peak values in histograms (A). Time interval on abscissa represents wave period.

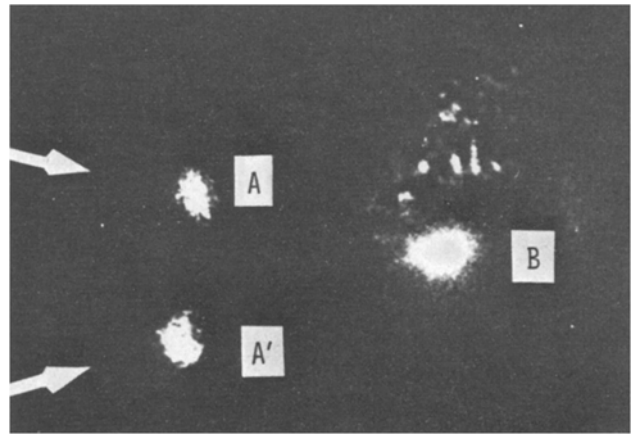

Fig. 3. A photograph of laser beams in the visual field of the disc covered lung surface during actual measurement. Spots A and A′ are caused by the reflections of the dual incident laser beams at the disc surface. Spot B is the probing area on an arteriole where the dual beams are crossed. The diffuse small spots are caused by the scattered laser showing vaguely the curvature of the alveolar surface.

central portion of the visual field can be received. Apart from these spots, the dual beams were crossed in the arteriole, causing unpaired single spot B. The lung surface can hardly be seen because the other part was non-illuminated.

Since the shot noise contains infinitely many waves having various frequencies, it cannot be filtered off by means of the electric filters. We have investigated a method of reducing the shot noise from the photomultiplier due to the reflecting light, so as to permit measurements of flow velocity in a backward scattering mode and found that the covering of the lung surface alienated the interface which reflected the incident beams strongly, far apart from the probing area. The high water content in the disc reduced the reflection at the rear surface of the disc. The reflection at the lung surface became weaker, probably because of the water trapped in the space between the rear surface of the disc and the tissue surface. Due to these advantages, the time-shared histograms in the covered lung surface showed a clear deviation in response to the cardiac cycle, in a sharp contrast to those in the non-covered lung surface.

Riva et al.[8,9] reported measurements of the retinal blood flow by means of the laser Doppler method in a backward reflection mode. Since, in their measurements, the laser beams proceed a long distance within the lens having a long focal length, the transparent tissue probably plays the function of alienating effectively the probing area from the interface between the air and tissues. As to the measurements in the other usual organs including the lung, the surface of the organs must be artificially covered in order to avoid undesirable shot noise due to the surface.

Although the blood flow in the arteriole of frog lung is briefly reported to be pulsatile[10], its quantitative measurements have not yet been reported. The present study shows that the flow velocity in the pulmonary arteriole begins to increase in an early phase of the cardiac cycle. This is probably due to the close situation of the lung to the heart. The N_2O uptake method[11] reveals that the blood flow of human pulmonary capillaries fluctuates strongly in response to cardiac events. The systolic flow attains 5 times as much as the diastolic flow. The blood flow velocity fluctuations, observed in the bullfrog, due to cardiac events, reach 25% of the mean value in the pulmonary arteriole, whose pulsatility is generally greater than in the capillary. The blood flow of pulmonary capillaries in the bullfrog is probably much smoother, and gas exchanges in the bullfrog could be made at a more continuous rate than in the human lung.

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Brain catecholamines and organ weight of mice genetically selected for high and low blood pressure[1]

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Summary. Statistically significant differences were found between the high and low genetically selected blood pressure lines for systolic blood pressure, norepinephrine content of whole brain, absolute heart weight, heart to b.wt ratio, kidney weight, kidney to b.wt ratio, and adrenal to b.wt ratio.

The physiological basis of the elevated blood pressure level in essential hypertension in man is for the most part unknown. There now exists a number of animal colonies in which selective breeding has provided animals with elevated blood pressures which may serve as animal models for human hypertension. There are currently 7 potential animal models among rodents, 6 rat colonies[2-7] and 1 mouse colony[8]. In each of these colonies the physiological basis for the elevated pressures is under investigation. This report describes some of the physiological and biochemical characteristics of the high and low blood pressure lines of mice compared to a random bred control.

Materials and methods. The 'high' and 'low' blood pressure lines used in this study were the result of 17 to 19 generations of selective breeding in a selection program designated BPI. Selection was begun in a base population derived from an 8-way cross among 8 inbred strains (LP/J, SJL/J, BALB/cJ, C57BL/6J, 129/J, CBA/F, RF/J and BDP/J) and was continued within closed lines. A concurrent control line propagated by random mating was maintained throughout the selection program. The development of these lines and the technique of indirectly measuring systolic blood pressure was described in a previous paper[8]. Briefly, systolic blood pressures were determined by occluding the flow of blood at the base of the tail and sensing the return of a pulse distal to the cuff when the pressure in the cuff was decreased (NarcoBio Systems Physiograph). The cuff width used was 25 mm, which work by Henry et al.[9] has shown to underestimate systolic blood pressure, and the diameter was 6 mm which is the appropriate size for mice over 23 g. The mice were restrained but unanesthetized and maintained at 37.5 °C during measurement. 5 readings were taken on each of 3 days during a period of 3–5 weeks when the mice were 100–150 days of age. In the 17–19 generation of selection the systolic blood pressure differed by 40–54 mm Hg between the high and low line.

Mice were sacrificed by cervical dislocation. Brains were rapidly removed and placed on ice before weighing. Individual brains were added to 1 ml 0.1 N $HClO_4$ containing 5×10^{-4} M $NaHSO_3$ and homogenized by sonification using