more prominent in the cytoplasm immediately surrounding the nuclei (Fig. 2).

Hair cells which were not injected, or injected with water, did not remain stained by fluorescent TMV-antibody after the cells were washed with phosphate-buffered saline. The validity of the fluorescent antibody identification of TMV was verified by first treating a base cell injected with TMV with TMV-antibody that was not conjugated with dye. These cells were then treated with fluorescent antibody. No definite specific fluorescence was recognized. Further evidence of the specificity of the fluorescent antibody reaction was found by the use of TMV inactivated by irradiation with ultraviolet light. By this means, TMV was inactivated so that it no longer produced lesions on pinto bean leaves but still retained its serological properties. The inactivated TMV was injected into hair cells. Within a few hours after injection, the nuclei sometimes showed a reaction to fluorescent antibody. However, the reaction disappeared 6 hours after injection, and no further antibody stain either on the nuclei or other cell components was seen at later times of observations.

When TMV-RNA was injected, no reaction of the type found with TMV was evident immediately after injection. However, 6 hours after injection a fluorescent reaction typical of TMV protein was detected in the nucleus and less frequently in the cytoplasm surrounding the nucleus. With longer periods after injection, the fluorescent antibody reaction had the same character found with infections induced by TMV.

Previous observations of cells already infected with TMV have all suggested that the nucleus takes part in the TMV infectious process. The results of this investigation with cells that were directly infected with either TMV or TMV-RNA confirm previous observations and provide rigorous new evidence to show that the nucleus is the first organelle in the injected cell to respond to the presence of virus. Furthermore, the nucleus is the first organelle in which TMV protein, as identified by a specific fluorescent antibody, can be detected. From results of biochemical studies, Reddi (*3*) stated that TMV may be synthesized in the nucleus. Only after the virus protein has been found in the nucleus can its presence be identified in the cytoplasm, and then only in areas that are closely adhering to the nucleus. These results are inconsistent with the observation by Schramm and Röttger (*4*), who demonstrated that the cytoplasm alone around the nucleus exhibited the specific fluorescence. Of particular interest is the observation that uninfectious TMV injected into hair cells still finds its way to the nucleus before it eventually disappears as a reactant to fluorescent antibody. Apparently, there is a highly specific interaction between TMV and the nucleus which signals the beginning of the TMV infectious process leading ultimately to the formation of new TMV nucleoprotein rods in the injected cell.

TOKUZO HIRAI
ATSUSHI HIRAI
Faculty of Agriculture, Nagoya University, Anjo, Aichi, Japan

References and Notes

1. H. Zech, *Planta* **40**, 461 (1952); J. G. Bald and R. A. Solberg, *Nature* **190**, 651 (1961); T. Hirai and S. G. Wildman, *Plant Cell Physiol.* **4**, 265 (1963).
2. H. Fraenkel-Conrat, B. Singer, A. Tsugita, *Virology* **14**, 54 (1961).
3. K. K. Reddi, *Proc. Natl. Acad. Sci. U.S.* **51**, 619 (1964); *Science* **144**, 566 (1964).
4. G. Schramm and B. Röttger, *Z. Naturforsch.* **14b**, 510 (1959).
5. We thank S. G. Wildman, Department of Botany and Plant Biochemistry, University of California, for reading this manuscript. This work was supported by NIH grant EF 00447-01. Contribution No. 94, Plant Pathology Laboratory, Nagoya University.

19 June 1964

Circulatory Adaptation to Diving in the Freshwater Turtle

Abstract. *The heart of the freshwater turtle has a functional ventricular septal defect. In ambient air there is some shunting of blood from the left to the right ventricle through the defect. During prolonged diving or N_2 inhalation, the shunt is reversed and blood from the tissues bypasses the lung and enters the aorta directly. This adaptation appears to be based on the exclusive use of anaerobic glycolysis as an energy source during prolonged diving.*

The circulation and external ventilation of vertebrates must be geared to intracellular metabolic requirements. The freshwater turtle is able to dive for extended periods in the absence of molecular oxygen (*1*). The fundamental adaptation which permits this is the animal's ability to exist for prolonged periods on energy derived solely from anaerobic glycolysis (*1*, *2*). In this report we describe an unusual circulatory adaptation which allows a more effective supply of substrates for anaerobic metabolism of the tissues.

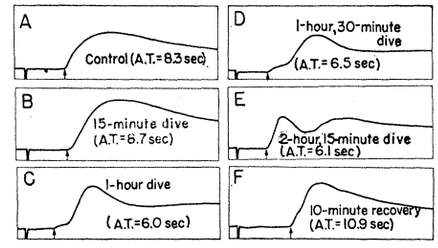

Fig. 1. Cardiac shunt progression. The effect of diving on dye-indicator curves obtained from the freshwater turtle. The time between injection of the dye and its appearance in the arterial system (↑) is indicated by A.T. (appearance time).

Freshwater turtles, *Pseudemys scripta elegans* and *floridana*, weighing between 1 and 2 kg, were used for our studies. A total of 40 experiments were conducted. Preliminary investigations of the anatomical features of turtle heart, aortae, and pulmonary arteries were made by direct inspection after appropriate dissection.

The pathways of blood flow were determined by use of dye indicator techniques (*3*). The Gilford continuous, automatic densitometer (*4*), commonly used for cardiac output measurements in man, was modified so that dye indicator studies could be performed in the turtle. The indicator dye used was indocyanine green. Peripheral arteries and veins in the neck were isolated and intubated with polyethylene catheters (PE 50). For the documentation of right to left cardiac shunts the bolus of dye was injected into a peripheral vein and the appearance times and contours of the time-concentration curves were monitored in arterial blood. For the documentation of left to right cardiac shunts the dye bolus was injected directly into the left ventricle after removal of the plastron, and indicator-dye concentrations were monitored in arterial blood. Heart rates were obtained from arterial pressure tracings. Experiments were performed in ambient air, during diving, during the inhalation of 100 percent N_2, and after the intravenous injection of 20 mg of NaCN.

The heart of the turtle has four chambers. As has been reported for all reptiles (except Crocodilia) the intra-

ventricular septum between the right and left ventricle is incomplete (*5*). The right and left ventricles have a potential communication through this large ventricular septal defect located in the anterior part of the intraventricular septum. At the base of the pulmonary conus is a valve-like cartilaginous structure not previously described. When this structure approximates the septal defect, then the two ventricles are anatomically discrete. When this structure is pulled away from the septal defect, then free mixing of right and left ventricular blood is possible. The origin of each main stem pulmonary artery is invested in a cartilaginous–smooth muscle coat.

Figures 1 and 2 show typical examples of curves obtained from turtles in ambient air and during diving immediately after the injection of dye. In ambient air there is a relatively long interval between venous injection of the dye and the appearance of dye in arterial blood. Also, the dye is washed out of the arterial system at a relatively slow rate as shown by the downslope of the control curves (Fig. 1*A* and Fig. 2*A*). A slow rate of disappearance has been correlated with the presence of left to right cardiac shunts in man (*3*). As diving time continues (1 hour, Fig. 1*C*) a second rise in the curve appears after a relatively short time. This rise is inscribed by blood which enters the aorta directly without passing through the pulmonary circulation (right to left shunt). As the dive continues, the amount of blood going directly from the venous side of the circulation to the arterial side increases, and ultimately (Fig. 2*B*) the entire cardia output may bypass the lungs completely. This occurs approximately 2 to 3 hours after diving. There is also an increasing steepness in the downslope, a finding which is consistent with a decreasing amount of blood which flows from the left to the right ventricle through the septal defect. Curve *F* in Fig. 1, obtained 10 minutes after the animal was returned to room air, shows a long interval between injection and appearance of the dye in the arterial system (no right to left shunt) and the reappearance of a left to right intracardiac shunt. It should be emphasized that the interpretation of these events does not depend on any analogy with dye indicator curves in mammals. These data demonstrate the development of a route of blood flow with a rapid time constant during diving.

Figure 3 shows that a similar sequence of events occurs as a result of exposure of the animal to the inhalation of 100 percent N_2. However, the administration of NaCN which inhibits O_2 utilization, resulting in high and not low blood oxygen tensions (*1*), produces no right to left shunting.

Figure 4*A* shows a typical example of a curve obtained after the injection of dye directly into the left ventricle with the animal in ambient air. The curve is biphasic, the primary rise representing blood going directly from the left ventricle into the aorta, the secondary rise being inscribed by blood going from the left ventricle through the ventricular

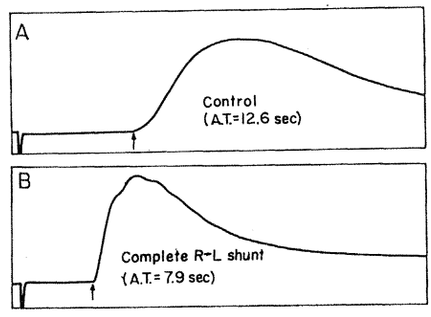

Fig. 2. Effect of diving on dye-indicator curves. The control (*A*) shows a 12.6-second interval between injection and appearance of dye in the arterial system. During a dive the interval is reduced to 7.9 seconds (*B*).

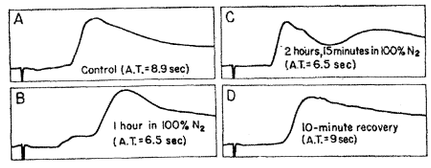

Fig. 3. Effects of N_2 on dye-indicator curves.

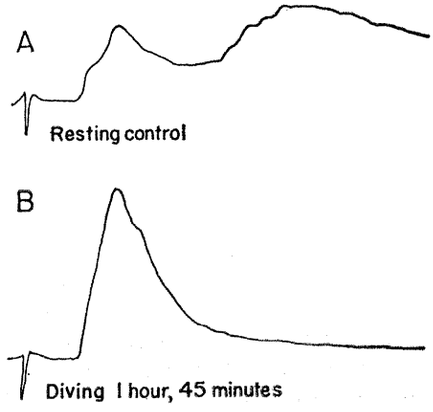

Fig. 4. Typical curves obtained after injection of dye into left ventricle.

septal defect through the pulmonary circulation and then into the aorta by means of the left ventricle. After diving, there is only a single rise in the curve (Fig. 4*B*). Thus, the biphasic curve with the animal in ambient air is not related to an increase of left ventricular pressure during dye injection. These data document the presence of a left to right intracardiac shunt while the animal is in ambient air and the disappearance of this left to right shunt during diving.

The heart rate remained essentially unchanged during diving. Thus, bradycardia, which is an important aspect of the adaptation to prolonged diving in aquatic mammals (*6*), does not occur in the freshwater turtle.

The pattern of adaptation to prolonged submersion in diving mammals such as the seal have been clearly delineated by a number of workers (*6*). This adaptation consists in part of generalized arterial constriction of all vascular beds except the brain. Thus, available O_2 is conserved for use by the central nervous system. The obligatory requirement for O_2 by mammalian brain for the preservation of cerebral function and structure is well known. In the case of turtle brain, anaerobic glycolysis is capable of maintaining integrity for long periods (*1*). Thus, there is no particular necessity for preserving O_2 supply to the brain. Continued blood supply to the tissues serves the purpose of transporting substrate for anaerobic glycolysis. However, when O_2 is no longer available in the lung there would appear to be no advantage in continuing the pulmonary circulation. Our studies demonstrate that, indeed, under these circumstances blood is shunted away from the lung and that pulmonary circulation ceases.

The stimulus for the development of the right to left intracardiac shunt appears to be a function of blood or tissue oxygen tension, since this shunt is produced by diving and N_2 inhalation but not by the administration of cyanide. The exact mechanics which produce the right to left shunt are not clear. Presumably, the development of the shunt is associated with increasing resistance to flow through the pulmonary artery. Whether the unusual cartilaginous–smooth muscle structures located at the origin of the main stem pulmonary arteries participate in the increasing pulmonary vascular resistance is not known.

It is also not clear how the valve-like

structure located close to the ventricular septal defect specifically operates to permit free flow of blood from right ventricle into the aorta. However, the development of a right to left shunt during diving in the turtle is a clear example of a circulatory adaptation which ensures provision of the optimum requirements necessary for metabolism.

J. EUGENE MILLEN
H. VICTOR MURDAUGH, JR.
CATHERINE B. BAUER
EUGENE D. ROBIN

Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; Department of Medicine, University of Alabama Medical Center, Birmingham; and *Mount Desert Island Biological Laboratory, Salisbury Cove, Maine*

References and Notes

1. E. D. Robin, J. W. Vester, H. V. Murdaugh, Jr., J. E. Millen, *J. Cell. Comp. Physiol.*, in press.
2. D. Belkin (abstract) *Physiologist* **5**, 105 (1962).
3. P. S. Hetzel, H. J. C. Swan, E. H. Wood, in *Intra Vascular Catheterization*, H. A. Zimmerman, Ed. (Blackwells, Oxford, England, 1959), p. 539.
4. Gilford Instrumentation Laboratories, Inc., Oberlin, Ohio.
5. E. S. Goodrich, in *Studies on the Structure and Development of Vertebrates*, E. S. Goodrich, Ed. (Dover, New York, 1958), p. 557.
6. P. F. Scholander, *Harvey Lectures, Ser. 57* **1961-62**, 93 (1962).
7. This work was supported by research grant HE-05059 from the NIH and by a research grant from the Tuberculosis League of Pittsburgh.

9 June 1964

Specificity of Potassium-Activated Phosphodiesterase of Escherichia coli

Abstract. *A potassium-activated phosphodiesterase that hydrolyzes polyribonucleotides to 5′-mononucleotides has been purified approximately 600-fold from extracts of* Escherichia coli *B. The purified enzyme appears to be specific for single-stranded polyribonucleotides: helical forms are not hydrolyzed, nor do they inhibit the hydrolysis of single-stranded chains.*

The usefulness of nucleases that show specificity for the conformation (or secondary structure) of their DNA (*1*) substrates has been widely demonstrated (*2, 3*). In studies with RNA, pancreatic ribonuclease has been used as an indicator of helical configuration (*4*) although its specificity for attacking single-stranded polyribonucleotides is not definitive. In addition, polynucleotide phosphorylase was shown to be relatively inactive on helical polyribonucleotides (*5*). We would now like to report that the potassium-activated phosphodiesterase of *Escherichia coli*, recently described by Spahr and Schlessinger (*6*), shows a clear-cut specificity for single-stranded polyribonucleotides. The usefulness of the enzyme for studies of polyribonucleotide structure as well as the possible physiological significance of its specificity will be discussed.

The enzyme preparation described by Spahr and Schlessinger (*6*), a crude extract from which ribosomes were removed by centrifugation, hydrolyzed polyribonucleotides, including polyU and the messenger RNA of bacteriophage T_2, to 5′-mononucleotides. Thus the enzyme was distinguishable from the previously described ribonuclease of *E. coli* (*7*) which is found bound to ribosomes and produces 3′-mononucleotides. At the time of the Spahr report (*6*) we were engaged in the purification of a nuclease present in the supernatant fluid of extracts of *E. coli* B that had been centrifuged at 105,000*g*; the properties of that enzyme led us to conclude that it is the same as the potassium-activated phosphodiesterase described (*6*).

We have now purified the enzyme approximately 600-fold. The hydrolysis of C^{14}-labeled polyA was used to measure enzyme activity, one unit of enzyme being equivalent to the hydrolysis of 1 μmole of polyA to acid-soluble material per hour. Polymer concentrations are given as mononucleotide (or phosphate) equivalents. The optimum assay conditions are similar to those reported previously (*6*), namely, 1.5m*M* Mg^{++}, 0.1*M* KCl, *p*H 7.5. Approximately two-thirds of the activity of the initial crude extract (20,000*g* supernatant of an extract of sound-treated *E. coli* B) is apparently lost upon treatment with protamine sulfate or removal of the ribosomes by centrifugation at 100,000*g*. As yet, we have been unable to account for these missing units. The best preparation we obtained was purified about 600-fold compared to the supernatant fluid resulting from protamine sulfate treatment (*8*) and it had a specific activity (units per milligram of protein) of 2180. The purified enzyme showed no detectable phosphatase activity at *p*H 4.7, 7.5, or 9.0, with 5′-AMP as substrate. Under the conditions used we would easily have detected activity equivalent to one-thousandth of the phosphodiesterase activity. The maximum possible contamination with polynucleotide phosphorylase is of the same order of magnitude. With this preparation, as well as with less highly purified enzyme, the only product detected upon hydrolysis

Table 1. The effect of nonsubstrate polynucleotides on polymer hydrolysis. Reaction mixtures (0.1 ml) contained 0.1*M* tris, *p*H 7.6; 1.5m*M* $MgCl_2$; 0.1*M* KCl; 1.2 μmole of polyU or 2.2 μmole of polyA per milliliter; and 1.3 enzyme units per milliliter (hydroxylapatite fraction, specific activity 2180). Additions per milliliter were as follows: *E. coli* sRNA, 20.6 OD_{260} units; yeast sRNA, 19.4 OD_{260} units; *E coli* DNA, 0.93 μmole P in the polyU experiment, and 0.68 μmole P in the polyA experiment; and polyI, 2.8 μmole. The reaction mixtures were treated as described in the legend to Fig. 1. The results are expressed as millimicromoles of mononucleotide hydrolyzed per reaction per 20 minutes.

Substrate	Addition				
	None	*E. coli* sRNA	Yeast sRNA	*E. coli* DNA	PolyI
PolyU	18			18	20
PolyA	42	44	41	38	

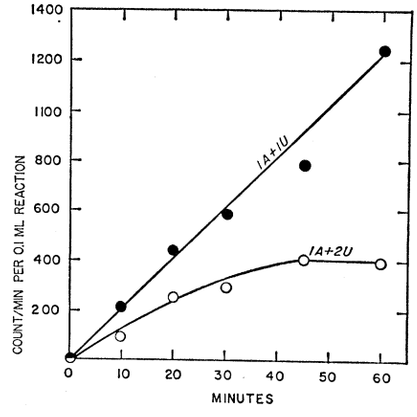

Fig. 1. Hydrolysis of complexes of polyA and polyU. Each reaction mixture contained 0.1*M* tris, *p*H 7.6; 0.1*M* KCl; 1.5m*M* $MgCl_2$; 0.1 mg of bovine serum albumin per ml; 0.76 enzyme unit (DEAE-cellulose chromatography fraction, specific activity 570) per milliliter of reaction mixture; and 1.13 μmole of polyA-C^{14} (specific radioactivity 27,300 count/min per μmole) per milliliter. They also contained polyU-C^{14} (44,300 count/min per μmole) as follows: 1) Closed circles, A + U experiment, 1.16 μmole; and 2) open circles, A + 2U, 2.32 μmole/ml. All reactions were carried out at 37°C. At the indicated times 0.1 ml portions were removed. Precipitation of undigested polymer was effected by two volumes of cold ethanol in the presence of unlabeled carrier RNA and 0.1*M* NaCl (*6*). Portions of the supernatant were counted. Results are expressed as count/min of soluble product per 0.1 ml of reaction.