as separate lethal mutations, the differential effect of this mutagen on survival and on suppressor mutations to streptomycin independence could be considered analogous to the differential mutagenic effect of a mutagen on different genes, as observed by Demerec (*6*).

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Measurement of Regional Blood Flow by Indicator Dilution

Abstract. Appropriate selection of injection and collection sites permits quantification of certain regional blood flows by the single-injection, indicator-dilution method. Quantifying formulas are derived, and application of the method to several regional beds is described.

The indicator-dilution method (*1*) is used extensively to measure cardiac output but has had limited application to regional flow. Following rapid venous or central injection of an indicator, successive curves of rising and falling indicator concentration are inscribed at an artery, representing initial circulation and recirculations. If the amount of indicator injected (I) is known, and if the area (A), which is the product of the average concentration (C) and the duration of the curve (T), is determined for the extrapolated semilogarithmic graph of the initial curve, then the flow (Q) is calculated from the following conventional formula:

$$Q = I/CT = I/A \qquad (1)$$

It follows that if, after central injection, the amount of indicator entering a given vessel were known, flow through that vessel could be calculated. Thus, for the vessel R:

$$I_R = Q_R C_R T_R \qquad (2a)$$

and

$$Q_R = I_R/A \qquad (2b)$$

where A is the area under the local curve ($C_R T_R$) or under any simultaneous peripheral dilution curve, all such areas being equal.

If R is the single afferent or efferent for a region, knowledge of I_R permits calculation of the regional flow. I_R is proportional to the unknown flow and has no other physiologic determinants. If, however, a natural or designed measurable supplement to Q_R produces a measurable alteration in A, solution is possible by means of simultaneous equations.

If any vessel or chamber V receives blood from several sources, among them the complete flow for region R, and if I_R is delivered prior to indicator I_X from other sources, an early curve, representing Q_R, will be inscribed, following mixing, at sites distal to V. The average concentration of the indicator (C_V) during inscription of this early curve is a function of the indicator (I_R) and the flow ($Q_R + Q_X$) that traverses V during the time interval T. Thus:

$$I_R/(Q_R + Q_X)T_V = C_V \qquad (3)$$

Combining Eqs. 2 and 3, we have:

$$Q_R A = (Q_R + Q_X) C_V T_V \qquad (4)$$

$C_V T_V$ is the area under the early curve. Therefore:

$$Q_R A = (Q_R + Q_X) A_V \qquad (5a)$$

or

$$Q_R = \frac{(Q_R + Q_X)}{A/A_V} = \frac{Q_X}{(A/A_V) - 1} \qquad (5b)$$

A is the area under a conventional arterial curve. Therefore, whenever a discrete A_V is obtained, the Q_R responsible for it can be quantified as a function of Q_X or of $Q_R + Q_X$. The latter, moreover, is a measurable quantity whenever V is a cardiac chamber, since $Q_R + Q_X$ must then become the output of one of the ventricles, and, in certain instances, Q_X may be a measurable fraction of a ventricular output. Thus, one can measure flows which empty into a cardiac chamber to produce a dilution curve distinct from those of general circulation and recirculation.

This principle is applicable to at least four systems:

1) With left-to-right shunts—through atrial septal defects, for example—shunted blood (Q_R) joins Q_X to produce pulmonary flow, which can be estimated from a peripheral arterial dilution curve after injection into the pulmonary artery, while Q_R produces an early curve from the right ventricle. The ratio of the area of the early right ventricular curve to the area of the systemic arterial curve equals the fraction of pulmonary flow traversing the shunt.

2) After proximal aortic injection, the earliest curve expected from the left ventricle, in the absence of valvular regurgitation, is that of the indicator completing its first circulation. However, physiologic shunts exist in the form of systemic-pulmonary (chiefly bronchopulmonary) communications through which a portion of the left ventricular output returns to the left atrium without traversing systemic great veins and the right heart. This pulmonary collateral flow (Q_R) plus right ventricular output (Q_X) equals systemic flow, which is estimated from the peripheral arterial dilution curve, while Q_R produces an early curve from the left ventricle. The ratio of the area of the early left ventricular curve to that of the systemic curve equals the fraction of the left ventricular output traversing pulmonary collateral channels.

3) The first indicator to appear at the pulmonary artery after left atrial injection should be that traversing the short, rapid, low-volume pathway through the coronary sinus into the right atrium. The systemic output is estimated from a peripheral arterial curve, while the early pulmonary artery curve is proportional to coronary sinus flow, and the ratio of the area of the latter curve to that of the former expresses coronary flow as a fraction of systemic output.

4) With mitral regurgitation the flows converging upon the left atrium are the forward flow (Q_X) and the backflow (Q_R). After left ventricular injection, the early curve from the left atrium will be proportional to Q_R, while the conventional systemic arterial curve will be proportional, not, as in the situations cited above, to the sum of Q_R and Q_X, but to Q_X alone, the forward flow. Since the measured general flow is a fraction of, and not the entire, ventricular output, the version of Eq. 5 required is that in which Q_R equals the general flow divided, not by the ratio of the general to the regional area, but by that ratio minus 1.

In addition, one can infuse Q_X exogenously, as a known volume, into the arterial inflow to any part. If, then, following central injection and mixing of the indicator, a turbulence exists at or distal to the infusion site sufficient to produce mixing between the arterial blood and the infusate, a collection downstream will reveal a proportionally altered dilution curve. The ratio of the area of the latter curve to that obtained at another peripheral vessel will give the fraction of systemic output traversing the local bed. This has obvious implications for measurement of flows in organs and limbs.

Although the early appearance of dye at sites upstream from the site of injection has been used qualitatively to detect the presence of left-to-right shunts and mitral regurgitation (*2*), quantification of these or of other flows by comparison of the areas of modified regional curves with general systemic curves has not, to our knowledge, been reported.

The practical applicability of this

principle has been established by us chiefly in the study of pulmonary collateral flow (*3*). The formula has made it possible to quantify observations of such flow made earlier by one of us (L. C.) at Yale University. Those investigations—the earliest in which this experimental design was applied to the study of a physiologic flow—have been extended in this laboratory to human subjects. In several instances we have measured total pulmonary collateral flow by this and an independent indirect method, with agreement between the two results. Our studies of shunt flows and of mitral regurgitation are in progress and are promising.

Since indicator dilution methods are not confined to cardiovascular physiology, it is conceivable that the principle here described will prove useful in other problems of flow measurement.

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Function of Giant Mauthner's Neurons in the Lungfish

Abstract. Unit spikes were recorded from the spinal cord of the lungfish *Protopterus* and were identified with Mauthner's axon. With these spikes occurred nongraded tail flips suggesting startle responses. The tail flip and the giant spike resulted from certain forms of jarring and prodding. The conduction velocity for the slightly myelinated 45 μ diameter fibers was 18.5 m/sec.

The Mauthner's neurons of teleosts and urodeles have been the subject of many anatomical and embryological studies (*1*). Speculations about their function have had little physiological basis, and electrical recordings presumably from these neurons (*2–4*) have shed no real light on their normal role in the animal. Berkowitz (*4*) was the first to give some experimental grounds for assuming a special startle-response function in a carp, *Cyprinus,* and Retzlaff (*5*) reported comparable results which could be similarly interpreted. Berkowitz showed that physiological (tapping) stimulation of a certain type elicited sharp tail flips and high-velocity cord potentials, and that electrical stimulation of the cord just at the threshold point for such post potentials gave the same tail flip. The potentials were not all-or-none but presumably involved both Mauthner's and some other large fibers of the medial longitudinal fasciculus. Retzlaff recorded from what was doubtless the Mauthner's cell and saw similar tail jerks, but his results, obtained with semimicroelectrodes, do not permit the conclusion that the response is all-or-none activity in a single unit, though the Mauthner's spike and tail jerk were one-to-one. It seemed desirable to show more clearly whether a stereotyped, abrupt, maximal, twitchlike, normal movement is mediated by activity of the Mauthner's axon alone.

That *Protopterus* would be especially suitable for such experiments was suggested when Smith (*6*) called attention to the large size of the Mauthner's axons in this lungfish. In *Protopterus* this pair of fibers in the ventral columns of the spinal cord is truly giant and, therefore, may be expected to be accessible to selective electrical stimulation by virtue of a relatively low threshold and to single-fiber recording from the intact cord. The experiments described in this report (*7*) were performed on three 20- to 30-cm fish.

On the assumption that giant fibers mediate startle responses, attempts were made with various types of stimulation to produce sudden, single, stereotyped, large-magnitude movements. Individuals varied considerably in their responsiveness, but, although they were usually sluggish, all of them under some kinds of stimulation showed this type of reaction, confined to a sharp flexion of the tail. The response was produced by deep probing with a needle, by dropping the dish containing an animal onto the table, and by massive electrical stimulation of the body surface or of the exposed spinal cord. It occurred more readily in one specimen when the fish was placed on a dry surface. It was sometimes repetitive, alternating from side to side (compare Retzlaff's simultaneous stimulation of both VIIIth nerves). Weak vibration from a tuning fork, a jarring of the aquarium, strong light, or disturbances of spatial orientation failed to elicit the response.

Application of single electrical shocks to the dorsal surface of the spinal cord, above a sharp threshold, caused the same type of movement. The magnitude of the response to a single shock was nearly as great as that resulting from tetanizing frequencies. Electrical recordings from a second region of the cord showed two main components of activity following electrical stimulation. The first was a single all-or-none, sharp-threshold spike having an apparent conduction velocity of 18.5 m/sec (at about 20°C). The second was a complex wave which was graded in magnitude with respect to stimulus intensity and which was conducted at apparent velocities of between 4.1 and 1.3 m/sec. Spikes similar in form to the first wave were seen also during stimulation of the body surface by the same probing and electrical shock which had been found to give the startle response. These spikes were many times larger than any which occurred during ordinary locomotion and were always accompanied by the flip of the posterior trunk and tail described above.

The results indicate that the Mauthner's neuron in the lungfish, by itself, can mediate a special type of prompt, nongraded tail-flip response which may be compared with the startle response of many invertebrates possessing giant fibers (*8*).

The velocity of 18.5 m/sec is surprisingly low in comparison with the 35- to 40-m/sec velocity at 5°C (*5*) and the 50- to 60-m/sec velocity at 10° to 15°C (*2*) in *Ameiurus*—a fish in which Mauthner's fibers measure 22 to 43 μ in outside diameter—and with 80 m/sec (at 23°C) in *Parasilurus* (*3*) and 55 to 63 m/sec (at 20° to 25°C) in *Cyprinus* —fish in which Mauthner's fibers measure 55 to 65 μ (*4*). Random sections through the cord in the same specimens of *Protopterus* showed Mauthner's fibers of 45 μ in formalin-fixed preparations. These fibers had myelin sheaths accounting for no more than 3 percent of the total diameter, as compared with 50 percent in *Ameiurus* (*2*). There can be little doubt that the fiber stimulated and recorded from is Mauthner's, in view of the great discontinuity in size between it and the next largest fibers—a feature in which *Protopterus* stands out.

The large number of input sources to this cell which have been histologically identified appear to result in the simplest of outputs—one or a few impulses, or nothing. The most conspicuous source of input is vestibular, but simple displacement, tilting, or acceleration are apparently inadequate to fire the cell. The only physiological form of adequate stimulation found in these specimens, under the conditions of these experiments, was a severe jar. Possibly this represents an intense and synchronous activation of certain elements of the VIIIth nerve, similar to Retzlaff's electrical stimulation of the same nerve. By analogy with carp, earthworm, crayfish, and squid, it may be expected that under other conditions of set or readiness a much weaker stimulus would be adequate. It seems likely