Specific chemical keys or stimulation techniques may be necessary to isolate and trace separate functional circuits, but such tools seem to be rapidly becoming available (*1, 5–7*).

In this connection, our recent emphasis has been on testing animals under conditions which maximize the possibility of measuring changes related to any primary drive during chemical stimulation of the brain. One animal has been found that consistently responds to injection of carbachol by drinking, to injection of noradrenalin by eating, and to injection of a soluble steroid by building nests. All three chemicals have been applied to the same rhinencephalic locus at the junction of the area of the diagonal band of Broca and the medial preoptic region, and all three effects are specific with respect to the chemical or chemical family implicated.

Another aspect of the data worth discussing is the finding that some of the positive areas produce a significantly greater drinking response than others (see Table 1). The density of selectively sensitive neurons in an area, or the precision of delivery to a positive locus, may be involved, but other possibilities suggest themselves. All but one of the positive areas (reuniens) for which an attendant water intake of 20 cm^3 or more is frequently recorded are in the hippocampus or directly project to it. It is thus possible that sustained hippocampal afterdischarge is responsible for the prolonged and accentuated drinking that follows cholinergic stimulation of these areas. Electroencephalographic recording should reveal any correlations between separate types of hippocampal electrical activity and drinking. A second, but currently less likely, hypothesis is that the hippocampus normally functions as an inhibitory part of the drive system and that carbachol produces local seizures which temporarily disrupt hippocampal function and indirectly increase drive. Again, it should be possible to select between hypotheses by utilizing electroencephalographic techniques with animals prepared with multiple combinations of chemical and electrical implants.

Finally, it should be stated that the correlation between drinking and cholinergic stimulation of the brain is remarkably specific. It is true that we have found five cases in which injection of carbachol increased both eating and drinking, and one in which injection of either carbachol or strychnine consistently led to marked increases in food and water intake, but such cases or loci are quite rare. Animals injected with carbachol in the designated brain areas show a highly selective water-ingestion response and typically ignore stimuli allied to other primary drives for at least 20 to 30 minutes after drug injection. Such facts appear to weigh against the possibility that random firing or seizure activity in the limbic system underlies the response and that no true circuit is being traced. Indiscriminate neural firing would be expected to disrupt integrated response, or to influence a number of drives, rather than to selectively increase drinking.

In summary, the data of this study seem of particular interest because of the implication that a functional neural circuit can be traced through a selective sensitivity to a chemical agent, or to a particular range of concentration of that agent. Our own research (*7*) and that of others (*1*) has previously implicated only single or isolated loci, with little indication that entire circuits or their synaptic interconnections might be biochemically distinct. In addition, the new evidence, coupled with other recent data from studies of chemical and electrical stimulation of the brain, suggests the probability that relatively parallel neural circuits coursing through the limbic system and associated brain areas underlie the mediation of the primary drives (*8*).

ALAN E. FISHER
JOHN N. COURY
Department of Psychology,
University of Pittsburgh,
Pittsburgh, Pennsylvania

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3 August 1962

Quantitative Analysis of Blood Circulation through the Frog Heart

Abstract. Analysis of the oxygen content of blood in the frog heart and its major vessels has shown that in *Rana pipiens* the carotids receive primarily left atrial blood, which is highly oxygenated, whereas the pulmocutaneous vessels receive blood almost exclusively from the right atrium. Only the aortas receive blood subjected to considerable mixing.

In the anuran heart, blood from the left and right atria enters a single ventricle as two separate streams. This blood, after passing through a divided conus arteriosus, is distributed to three bilateral arterial trunks: the carotids, aortas, and pulmocutaneous vessels. Despite the absence of a ventricular septum, Brücke (*1*) postulated that the oxygenated left atrial stream and the less-oxygenated right atrial stream remain nearly separate when passing through the ventricle, that the more-oxygenated blood enters the carotids, mixed blood enters the aortas, and that the less-oxygenated blood enters the pulmocutaneous arteries. Vandervael (*2*) observed the passage of India ink through the ventricle and arterial trunks of *Rana temporaria* by transillumination and concluded that the blood leaving the heart had been thoroughly mixed in the ventricle. Simons (*3*), who also followed the passage of a dye injected into *R. temporaria,* and Sharma (*4*), on the basis of anatomical studies on *R. pipiens,* concluded that the blood entering the ventricle from the atria is selectively distributed to the arterial trunks. The degree to which this separation or mixing of oxygenated and less-oxygenated blood occurs in the ventricle and conus arteriosus is therefore controversial.

In the experiment reported here, oxygen occurring naturally in the blood was used to trace the distribution of the returning blood through the heart into the major arteries. Continuous breathing activity throughout the experiment was assured by only spinal pithing the frog, thus preventing injury to the nerve innervating the pharyngeal respiratory pump. A slit in the body wall was then made, extending about an inch caudad and craniad from the sternum, which itself was split lengthwise. If bleeding occurred, thrombin was applied to the cut surface and the amount of bleeding was thus kept to an estimated 0.05 ml or less. Samples of 0.15 to 0.3 ml of blood were withdrawn from the desired vessel with a No. 27 needle attached

Table 1. Mean percentage difference in oxygen content between the vessels compared.

Vessels compared	Change in O_2 content (%)	Standard error	No.
Right atrium and pulmocutaneous	120	6.3	11
Pulmocutaneous and aorta	160	12.6	12
Aorta and carotid	130	11.6	10
Carotid and left atrium	130	14.2	9

to a 1-ml tuberculin syringe which had been flushed with heparin anticoagulant (1000 U.S.P. units/ml). All oxygen analyses were made on sub-samples of equal size by the method developed by Roughten and Scholander (*5*).

To minimize the possible disturbance of pressure relationships within the circulatory system, blood was withdrawn at a rate approximating the flow in the vessels, as estimated by bulging or constriction of the vessel wall. To determine whether withdrawal of blood was causing a change in the distribution of blood by the heart, blood was also withdrawn from the aorta both 1 cm from the conus and distally, just proximal to the juncture of the left and right aortas, a point at which an amount of blood equal to that withdrawn would already be contained in the vessel. These proximal and distal readings were equivalent, and since all samples taken in any artery were at least 1 cm from the conus, it was assumed that withdrawal of blood did not significantly alter the flow through any vessel. It is conceivable that different mixing patterns could occur at the higher blood pressures found in unpithed frogs. However, in the course of the experiment, frogs in prime breeding condition with a high oxygen titer in the blood and an estimated high blood pressure, and commercially obtained frogs with low oxygen titer and low estimated blood pressure, showed a similar pattern of distribution for the oxygenated blood from the left atrium.

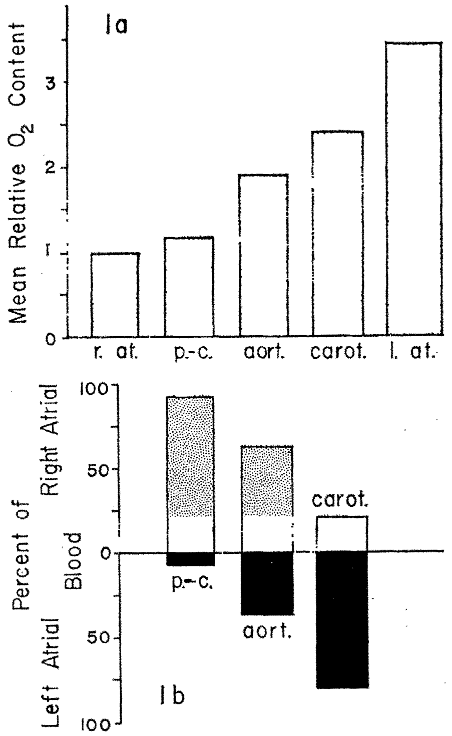

Fig. 1. Oxygen content in the vessels shown in *a* are multiples of the value for the right atrium; these values suggest mixing in the proportions shown in *b*.

So great a difference was found in the oxygen level of the blood in comparable vessels of different frogs that the results have been tabulated as a difference in oxygen content between different blood vessels of each individual frog (Table 1). On the basis of these data the oxygen contents of these vessels are compared directly as multiples of that in the right atrium (Fig. 1*a*).

If a separation of the blood streams within the ventricle is to be postulated, a consistent difference in the oxygen content of the vessels leaving the heart must be shown. The considerable difference in the oxygen content of the aorta and carotid arteries over that of the pulmocutaneous arteries indicates a directed and continuous, even though partial, separation of the atrial blood streams in the ventricle. On the other hand, since the oxygen content of the various arteries was always between the values for the two atria, some mixing of the blood in the ventricle must occur. If the difference in oxygen content between the vessels is considered, the degree of mixing between the left and right atrial stream can be quantified (Fig. 1*b*).

Blood entering the pulmocutaneous arteries is primarily right atrial, whereas that entering the carotid arteries, although mostly from the left atrium, contains some blood from the right atrium, showing that considerable mixing of the two streams has taken place. In the aorta, nearly two-thirds of the blood is from the right atrium and one-third from the left atrium. These results support the work by Simons (*3*) who stated that in anurans most of the left atrial blood enters the carotid and aortic arteries. Secondarily, Simons found an unequal distribution of oxygenated blood to the left and right aortic arches; unfortunately, these two vessels were not critically compared in the present study, since only the left side was sampled. Sharma (*4*) stated that in *R. pipiens* the aortic and carotid arteries receive only left atrial blood and the pulmocutaneous arches receive a mixed stream. The present study does not support this statement (Fig. 1*b*).

In *Rana pipiens* the carotids receive primarily left atrial blood which is highly oxygenated, whereas the pulmocutaneous vessels receive blood almost exclusively from the right atrium. Only the aorta receives blood subjected to considerable mixing (*6*).

KARL T. DELONG

Museum of Vertebrate Zoology,
University of California, Berkeley

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1 October 1962

Effect of Malathion Analogs upon Resistant and Susceptible Culex Tarsalis Mosquitoes

Abstract. The effects of minor variations in the malathion molecule upon the resistance of *Culex tarsalis* have been examined. By replacing the carboethoxy group by carbomethoxy, the 60-fold resistance to malathion is abolished. The results confirm the importance of carboxyesterase action in determining susceptibility to malathion.

The insecticide malathion [O,O-dimethyl *S*-bis(carboethoxy) ethyl phosphorodithioate] is toxic to most insects and of very low toxicity to mammals. It has been shown that this is due to the more rapid degradation of malathion in mammals, due to a carboxyesterase which hydrolyzes the $COOC_2H_5$ (carboethoxy) group (*1*). Recently Matsumura and Brown (*2*) have shown that when a strain of *Culex tarsalis* mosquito becomes resistant to malathion, it does so as the result of an increase in a carboxyesterase. Confirmation of the importance of carboxyesterase comes from the demonstration that when it is inhibited in vivo by treatment with compounds such as EPN