

the relationship of Haldane,  $[COHb] = 245 [O_2Hb] [CO] / [O_2]$ , and the normal hemoglobin dissociation curve (5). Although  $[CO]$  in the upper chamber was zero at the start, it rose during the experiment, and its average value during the 30- to 60-minute period was used. As  $[O_2]$  rose, the calculated equilibrated  $[COHb]$  difference across the film fell; the mean values were 87, 68, 64, and 47 percent for the helium, 7 percent  $O_2$ , 21 percent  $O_2$ , and 100 percent  $O_2$  experiments, respectively, in Fig. 2. Thus the facilitated CO transfer rises under conditions where the equilibrated  $[COHb]$  difference falls.

The ratio, facilitated flux / diffusion flux of CO, obtained experimentally, is significantly less than would be predicted from the data of Scholander (1) on  $O_2$ , assuming that facilitation is produced by the diffusion of hemoglobin + ligand, that chemical equilibrium exists at the film surface, and that the diffusion coefficients of hemoglobin and its compounds are the same. For example, when the  $[CO]$  in the lower gas chamber is 0.065 percent and  $[O_2]$  is zero, the difference in concentration of dissolved CO across the film is

$$0.065 \text{ percent} \times 0.018 = 0.000012 \text{ ml/ml}$$

In the absence of  $O_2$ , 0.065 percent CO will produce a COHb saturation of 97 percent (5). Therefore the difference in  $[COHb]$  across the film will be

$$0.97 \times 10.8/100 \times 1.34 = 0.14 \text{ ml/ml}$$

where 1.34 equals the total milliliters of CO bound per gram of Hb (5). Assuming that  $D_{CO}/D_{COHb} = 1/50$  (6, and as calculated from the data of Scholander, 1), the facilitated flux / diffusion flux should equal

$$0.014 / (0.000012 \times 50) = 233$$

From Fig. 2 the experimentally determined ratio is

$$\frac{(0.065 \text{ mm}^3/\text{min})}{(0.0028 \text{ mm}^3/\text{min})} = 23.3,$$

one-tenth that predicted.

One explanation of these findings is that the reaction velocities of CO and Hb are limiting the overall process and that chemical equilibrium does not exist between hemoglobin and the gases at the film surfaces. The equations describing steady state diffusion and reaction of CO in a layer of hemoglobin solution (7) are

$$-D_{CO} \frac{\partial^2 [CO]}{\partial X^2} = D_{COHb} \frac{\partial^2 [COHb]}{\partial X^2} \\ = l[COHb] - l'[CO][Hb]$$

$D_{CO}$  and  $D_{COHb}$  are the diffusion coefficients ( $\text{cm}^2/\text{sec}$ ) of CO and COHb respectively in hemoglobin solution;  $l$  ( $\text{sec}^{-1}$ ) and  $l'$  ( $\text{sec}^{-1}M^{-1}$ ) are the reaction velocity constants for the dissociation and association reactions respectively;  $[CO]$ ,  $[COHb]$ , and  $[Hb]$  are the molar concentrations of dissolved CO, carboxyhemoglobin, and reduced hemoglobin, respectively;  $X$  is distance in centimeters;  $l$  is not a proper constant in that it increases as  $[Hb]$  decreases (8).

It is impossible to obtain an analytical solution of the differential equations, and numerical methods are needed (7). It can be shown by approximate methods that the transfer of CO in these experiments is partially limited by the rate at which CO can dissociate from COHb and leave the upper surface of the film. Oxygen, by competing with the CO for the reduced hemoglobin, facilitates this process and increases the overall rate of transfer. Since there will probably be little reduced Hb in the layer,  $O_2$  will tend to dissociate from  $O_2Hb$  at the lower surface where the CO tension is higher, and to associate with Hb at the upper surface, where the CO tension is lower. This will result in a movement of  $O_2$  into the lower chamber in the absence of an  $O_2$  tension gradient between the gas chambers.

Not only have Scholander and his

associates pointed out an interesting phenomenon, but their work raises the question as to whether these, or thinner, layers of solution could not be used to measure chemical reaction velocities of biological interest (9).

M. MOCHIZUKI\*

R. E. FORSTER

Department of Physiology, Graduate School of Medicine, University of Pennsylvania, Philadelphia 4

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9. This work was supported in part by a grant from the Life Insurance Medical Research Fund.

\* On leave of absence from the Research Institute of Applied Electricity, Hokkaido University, Sapporo, Japan; Isaac Ott fellow of the department of physiology, Graduate School of Medicine, University of Pennsylvania.

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### Extrathyroidal Iodide Pump in Tadpoles (*Rana grylio*)

**Abstract:** Large amounts of iodide are accumulated in the gastrointestinal tract of tadpoles despite the presence of ligatures on the bile duct or esophagus or on both structures. These ligatures exclude the possibility that the iodine-131 tracer reaches the gastrointestinal tract by way of the bile or esophagus. The iodide in the gastrointestinal tract localizes largely in the stomach. The stomach therefore acts as an iodide pump and may be responsible for the maintenance of the iodide levels that are needed in metamorphosis.

The significance of the thyroid gland in amphibian metamorphosis has been well established (1). Swingle (2) also reported that iodide administered as either iodine or iodide was the active principle capable of inducing metamorphosis in the thyroidectomized as well as in the normal tadpole (3). The utilization of iodide by the animal is then a matter of importance since, even in the absence of the thyroid gland, the animal is said to achieve metamorphosis in the presence of adequate amounts of iodide in its environment

(2). Gudernatsch however has never confirmed the observation of a stimulatory effect of iodide or iodine on metamorphosis (4). The distribution of  $I^{131}$  in some tadpole tissues has been described, and the presence of  $I^{131}$  in the gut and pigmented tissues has been noted (5). No attempt was made to quantitate the  $I^{131}$  accumulated in these tissues (5).

In the present study *Rana grylio* tadpoles in the early hindlimb-bud stage were used. Three groups (ten tadpoles in each) were subjected, under tricaine (0.05 percent) anesthesia, to surgical procedures in which ligatures were placed on the esophagus near the cardiac sphincter, or on the bile duct, or on both the esophagus and the bile duct. In another experiment ligatures were placed around the esophagus and pylorus of ten tadpoles. Five tadpoles served as control groups for each experiment. Since the operated animals survived only 5 to 7 days after the ligatures were placed, the experiments were carried

Table 1. Localization of  $I^{131}$  in tissues of *Rana grylio* tadpoles treated by ligation of bile duct (BD), ligation of esophagus in the region of the cardiac sphincter (Es), simultaneous ligation of both bile duct and esophagus (BD-Es), and ligation of cardiac and pyloric regions of stomach (St). The tadpoles were immersed in carrier free  $I^{131}$  solution (0.3 to 0.5  $\mu\text{C}/\text{ml}$ ) for 70 hours.

Group	Activity (%)*							
	Tail	Torso	Skin	Intestines and contents†	Stomach	Liver	Kidney	Bile
Normal	3.6	2.0	5.3	77.3		2.8	3.1	5.9
Ligated BD	1.3	2.2	9.1	74.7		3.4	2.8	6.5
Ligated Es	3.3	5.4	13.3	61.6		6.2	5.8	4.4
Ligated BD-Es	2.3	4.1	12.6	56.8		5.5	14.0	4.7
Normal	1.1	1.7	5.0	13.7	73.9	1.3	1.5	1.8
Ligated St	0.9	2.3	4.0	4.0	75.9	3.3	2.6	6.3

\* Activity (%) = [(activity of  $I^{131}/100$  mg)  $\div$  (sum of activity of  $I^{131}/100$  mg in all tissues)]  $\times$  100. † The intestinal tract was emptied of its contents in some cases, and higher levels of iodide were found in the contents (7). The first four entries refer to the gastrointestinal tract. In the last two entries only the intestine is involved. For these two measurements the stomach and its contents were separated from the intestine and counted separately.

out on five animals from each group 2 days after they recovered from surgery.

The tadpoles were placed in a liter of tapwater containing between 150 to 250  $\mu\text{C}$  of  $I^{131}$ . The animals were left in this  $I^{131}$  water for 70 hours and then dissected after being anesthetized with a 0.1 percent tricaine solution.

Skin (stripped down and inverted from a small ring around the oral cavity to the tip of the tail), tail, digestive tract, liver, bile, kidney, and torso were collected from each tadpole in each group of the experiments. The torso included the brain, eyes, gills, gonads, fat bodies, heart, and so on. The bile was collected from the gall bladder, and its weight was determined from the difference in the weight of liver before and after the gall bladder was emptied. The radioactivity of each tissue was measured with a scintillation counter. The activity per 100 mg of tissue was calculated for each tissue, and the average activity of the similar tissues from the five tadpoles in each group was computed. The total activity for the average whole animal was arrived at from the activity per 100 mg for each tissue and the percentage activity of each tissue computed from the former value (6).

The data are illustrated in Table 1. The intestine and contents show a high percentage of the total iodide accumulated by the rest of the tissues (7). The route by which  $I^{131}$  arrives in the digestive tract must be via the blood stream since its passage into the digestive tract is not changed when ligatures are placed on the esophagus and the common bile duct. The second experiment demonstrated that the stomach, isolated by ligatures or in the terminal dissection procedure, contained about 95 percent or almost all

of the  $I^{131}$  previously found in the whole digestive tract. While no attempt is made to emphasize the statistical significance of the concentration of  $I^{131}$  in the tissues, the values were reproducible within about 15 percent and the greater effectiveness of the stomach in accumulating iodide was striking.

The accumulation of iodide in the stomach of the tadpole is probably incidental to its chloride pumping activity since the stomach forms hydrochloric acid and is reminiscent of the mammalian gastric mucosa (6). This pump may serve to concentrate, create a pool of, and recycle iodide. It thus may provide for the iodide needed during metamorphosis (8).

HARRY LIPNER  
SAMIRA HAZEN

Division of Physiology, Department of  
Biological Sciences, Florida State  
University, Tallahassee

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3. We have confirmed Swingle's observation that both iodide- and iodine-treated flours induce metamorphosis in thyroidectomized tadpoles. We have also found that the addition of iodide (potassium iodide) or elemental iodine to flour causes the appearance of thyroxine in an aqueous extract of the treated flour. The presence of thyroxine was shown with paper chromatography using two different solvent systems—butanol and acetic acid, and butanol, dioxane, and ammonia. It is therefore questionable that this experiment proves metamorphosis in the absence of a source of thyroid hormone in *Rana grylio*.
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6. Several independent series of such experiments have confirmed the validity of these data.
7. Approximately 14 percent of iodide in the digestive tract may be associated with the wall of the system. But since the contents were expressed and the tract was not washed, this figure is probably high.
8. This study was supported by a grant from the National Institutes of Health.

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## Sensory Deprivation:

### Its Effects on Human Learning

**Abstract.** The rote learning ability of human subjects under conditions of sensory deprivation for 48 hours was compared with results for a control group. The performance of the two groups was essentially the same. No significant facilitation or decrement in performance was noted, and in no instance were hallucinations, delusions, or other unusual phenomena reported.

Recent experimental studies (1) have indicated the variety of psychological effects which may occur in subjects under conditions of sensory deprivation, but few studies to date have been concerned with formal investigation of the effects of such conditions on learning in human beings.

In 1956 Vernon and Hoffman (2), reporting the effects on rote learning of confinement for 48 hours in a McGill type cubicle, concluded that learning was facilitated over the period of confinement, as indicated by a decrease in the number of trials required to meet the criterion. Such facilitation was not demonstrated in an extension of this study by Vernon and McGill (3) in 1957, as initially reported, but the conditions of the two studies were not exactly comparable. The investigation reported here is a further extension of the studies of Vernon and his associates (2, 3).

The paid, volunteer subjects were graduate and undergraduate students at the University of Miami. There were initially 18 subjects in the experimental group (six failed to complete the experiment) and 12 control subjects. The experimental cubicle, like that of Vernon and Hoffman (2) was 4 by 9 by 8 feet, lightproof and partially soundproof. It contained only a cot, the speaker for presenting word lists, and a two-way intercommunication system. The door of the cubicle contained a small one-way screen for monitoring the subjects' behavior. A shielded 20-watt bulb in the ceiling provided diffuse low-level illumination, and a large fan in the ceiling provided ventilation as well as a constant masking noise. The subject lay on the cot; he wore opaque goggles which permitted him to see light but interfered with patterned vision. Cotton gloves and cardboard tubes that extended from the elbows to below the finger tips minimized tactile stimulation.

Six word lists of equal difficulty, of 15 adjectives each [constructed from Hilgard's tables (4)], were presented