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5 July 1957

Physiological Response to Air Exposure in Codfish

When diving mammals and birds submerge they exhibit a series of characteristic phenomena. The heart rate slows down. Lactic acid accumulates in the muscles, but only a very little leaks out into the circulation, because blood flow through the muscles is strongly depressed. This part of the picture is virtually the same whether the animal is quiet or active during the dive. In the recovery after the dive, circulation increases, and the lactic acid from the muscles pours into the blood. These and other adjustments are strikingly developed in many diving mammals and birds and have been found, in a more or less pronounced degree, in every warm-blooded animal where such investigations have been made. Man also frequently develops a pronounced bradycardia during diving, even while swimming vigorously (*1*).

Unpublished experiments performed at Woods Hole, Mass., several years ago indicated that similar mechanisms operate in dogfish (*Squalus*) when they are taken out of water, and these observations led to the present investigation (*2*) on codfish (*Gadus callarias*) at the Biological Station at Drøbak, Norway.

Heart rate and lactic acid in blood and muscles were determined (i) when the fish were resting quietly in the water, (ii) when they were taken out of water and left struggling in air for 4 minutes, and (iii) when they were placed back in water for recovery. Heart rates were taken on the same fish throughout the sequence, but lactic acid samples were, in all cases, taken from different fishes and give therefore a statistical picture of the sequence. Heart rates were obtained by means of an electrocardiograph. Blood or muscle samples for lactic acid determinations were taken immediately after removing the fish from the water and were analyzed colorimetrically (*3*).

When the fish was taken out of water the heart rate dropped within a few seconds to half or less of normal (Fig. 1), and this bradycardia persisted even through violent muscular activity. When the fish was put back in water the normal pulse rate was rapidly restored.

In the muscles the lactic acid rose to a maximum during the air exposure and fell as soon as the fish was put back into the water. In contrast, the blood lactic acid remained low during the air exposure but rose in the recovery period and reached a maximum after some 5 to 15 minutes. As recovery proceeded, the lactic acid in blood and muscles slowly dropped back to normal (Fig. 1).

These events, produced by taking the fish out of water, are strikingly similar to those found when mammals and birds are submerged, and they very probably indicate the same sort of protective mechanisms against asphyxia—namely, a circulatory bypass of the muscles, which are thereby left isolated to operate on their own anaerobic resources. The bradycardia is very probably a direct consequence of the restricted peripheral blood flow, for at least in some diving mammals, the main arterial blood pressure does not drop during the bradycardia, which suggests that normal blood flow may be maintained in organs less capable of anaerobic functions than the muscles. The similarity of this mechanism to those present in diving mammals goes even further, inasmuch as bradycardia in the fish can be induced, just as in a seal, simply by frightening the animal. This "diving reflex" might seem to be an excellent idea for a flying fish, but how it could ever benefit a codfish is an evolutionary puzzle.

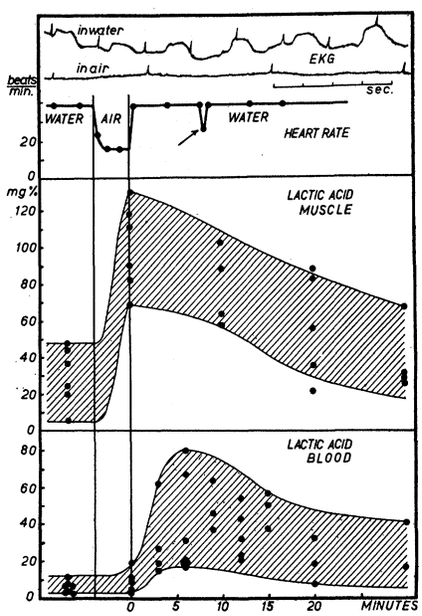

Fig. 1. Electrocardiograph tracings, heart rate, and content of lactic acid in muscles and blood of the codfish. The fish was taken out of water for 4 minutes. At arrow, hand was placed around submerged animal.

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24 June 1957

Irregular Maintenance Schedules and Drive

In animal experimentation the hunger drive is usually defined in terms of hours of food deprivation. Attention is rarely paid to the animal's prior history of deprivation. The deprivation history of an appetitive drive is controlled by means of a maintenance schedule—that is, the schedule of eating and deprivation intervals to which the animal is subjected over a period of time. If maintenance schedules affect subsequent behavior, then any variation from laboratory to laboratory may account for discrepant experimental findings.

Although it is known that irregular reinforcement profoundly affects animal behavior and is also a prominent feature of learning conditions outside the laboratory, almost nothing is known about the effects of irregular maintenance schedules. The present set of experiments represents a first attempt to study the effects of irregular food maintenance schedules on learning in the albino rat (*1*). It is expected that irregular schedules would elevate the drive effect of the deprivation interval used at time of testing.

In experiment 1, two groups of 70-day-old rats were placed on the following food maintenance schedules: a regular (R) group ($N = 5$) received 12 g of mash every 12 hours, and an irregular (I) group ($N = 6$) averaged the same amount of daily food intake but experi-

Table 1. Results of experiments 1 and 2. All p values refer to the significance of differences between group means based on t-tests; n.s. means not significant.

Item	Expt. 1					Expt. 2				
	R group ($N=5$)		I group ($N=6$)		p	R group ($N=10$)		I group ($N=10$)		p
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.	
				Acquisition						
Trials										
Original task	20.0	9.4	13.5	4.1	n.s.	19.1	13.9	22.5	12.3	n.s.
Reversal task	16.0	6.5	21.7	9.3	0.006	13.5	7.1	17.0	9.2	0.07
Errors										
Original task	23.4	11.3	17.0	5.7	n.s.	28.5	26.8	33.1	18.1	n.s.
Reversal task	20.0	9.3	29.8	15.1	0.002	14.8	9.9	23.7	16.7	0.14
				Disruption						
Vicarious trials and errors	4.9	4.1	1.2	1.2	0.03	7.4	6.8	2.7	3.3	0.04
Repeated errors	3.3	2.0	2.3	1.5	0.17	4.4	3.4	2.3	1.3	0.06
Latency	66.0	45.3	22.2	18.7	0.02	109.8	131.3	39.4	59.7	0.07

enced deprivation intervals varying from 12 to 48 hours. These schedules were maintained throughout the course of the experiment.

After 18 days the animals were introduced into a four-unit successive T-maze and trained to run a single alternation pattern (left-right-left-right). They were given five trials per night. Nights of running were spaced 48 hours apart, with a 72-hour interval every 6 days. This procedure gave the I group one 48-hour deprivation interval every 6 days, while at the same time enabling them to catch up with the R group on food intake and weight by the next night of running. All animals were run 12 hours deprived.

The R group received 12 g of mash 12 hours before running. The I group received from 12 to 18 g at this time. The exact amount was dependent on how much they had been deprived in the previous 48 hours, but it averaged 22 g. Care was taken to see that the weights of this group equaled or exceeded those of the R group at all times of running. In terms of food intake, the I group always received at least 12 g of mash more than the R group in the 24 hours preceding running. Therefore, on the basis of amount of food deprivation alone, at the time of measurement, the I group should be operating at a lower drive level than the R group.

When the animals reached a criterion of 95-percent correct choices on one night, the pattern was reversed to the mirror image, and they were required to learn this new pattern.

In experiment 2, all animals were given 50 overlearning trials before reversal. The R group ($N=10$) was maintained as in experiment 1, but the I group ($N=10$) had a slightly different schedule. Deprivation intervals ranged from 3 to 30 hours, and amount of mash per feeding varied from 4 to 22 g. By this procedure, not only did the I group receive the same average amount of food as the R group, but it also received the same number of feedings. Again, weights were comparable, the I mean slightly exceeding that of the R group. Measurements taken in activity wheels just before feeding showed no differences between the two groups (*2*).

It was expected that the I groups in both experiments would show behavior similar to that of animals tested under high-drive conditions. For purposes of such a comparison, previous data were available which compare 12-hour- and 36-hour-deprived animals (low- and high-drive groups) in two parallel experimental situations (*3*). Our R groups are equivalent to the previous 12-hour groups, while the I groups should show behavior more similar to that of the previous 36-hour groups.

Three dependent variables were utilized in the two studies: acquisition of original learning and of reversal learning and amount of response disruption occurring during the first trial of reversal. Table 1 summarizes the results of the present experiments. Table 2 compares differences between the R and the I groups with the differences obtained with the 12-hour and 36-hour groups. It can be seen from Table 1 that in the acquisition of the original task, the differences are not significant and are in opposite directions for the two experiments. During reversal learning, with analysis of covariance used as the control for original learning, the R groups showed faster acquisition than did the I groups in both experiments. Response disruption on the first trial of reversal was analyzed by means of three measures: number of vicarious trials and errors, number of repeated errors, and latency of the first correct response. The R groups made higher scores (showed more disruption) on all three measures than did the I groups, although only the vicarious trial and error and latency measures reach statistical significance.

From Table 2 it can be seen that the present results show some correspondence with the high- versus low-drive (*HD* and *LD*) experiments on errors during reversal learning and consistent correspondence on the various measures of response disruption. There was a lack of any agreement on acquisition of the original task. Thirty-six-hour-deprived animals consistently learned the original task slightly faster than did the 12-hour animals, whereas this was not the case with the I and R groups. One additional difference was noted. The 36-hour groups showed faster mean running speeds than did the 12-hour groups, whereas in the present study no consistent differences were found. In general, whenever significant differences were found between high-drive and low-drive animals, they were paralleled by corresponding differences between irregular and regular animals. Thus, irregularly deprived animals show more characteristics of animals in a high-drive state than do regularly deprived animals.

Table 2. Comparison of the effects of drive levels and maintenance schedules. The abbreviation n.s. means not significant.

Item	*LD* vs. *HD**		R vs. I	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
		Acquisition		
Trials				
Original task	n.s.	n.s.	n.s.	n.s.
Reversal task	n.s.	n.s.	$R < I$	$R < I$
Errors				
Original task	n.s.	n.s.	n.s.	n.s.
Reversal task	n.s.	$LD < HD$	$R < I$	n.s.
		Disruption		
Vicarious trials and errors	$LD > HD$	$LD > HD$	$R > I$	$R > I$
Repeated errors	n.s.	$LD > HD$	n.s.	$R > I$
Latency	$LD > HD$	$LD > HD$	$R > I$	$R > I$

* Data from *3*. All comparisons from this study are significant at the 0.05 level or better.

It is suggested that when drive level is defined in terms of hours of deprivation, the animals' prior history of maintenance schedules must be taken into account. Experiments on the effects of deprivation experiences occurring in infancy are now in progress.

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1 July 1957

Release of 5-Hydroxytryptamine by Benzoquinolizine Derivatives with Sedative Action

Previous investigations have shown that reserpine causes release of 5-hydroxytryptamine (5-HT) from various body depots (brain, intestine, and blood platelets). After a single injection of a large dose of reserpine, the 5-HT content of these organs decreased to values between one-fifth and one-tenth of the normal levels and remained low for several days. Among the *Rauwolfia* alkaloids, only those with tranquilizing action showed this effect. A series of centrally acting drugs belonging to other chemical groups did not influence the 5-HT content of the brain (*1*).

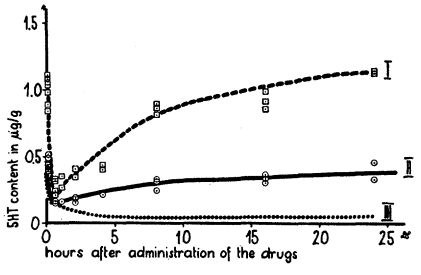
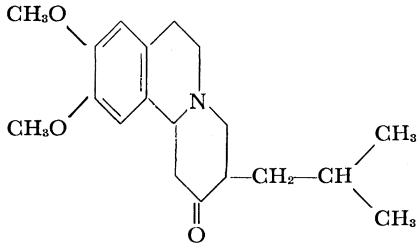


Fig. 1. Effect of compound I and reserpine on the 5-hydroxytryptamine (5-HT) content of brain. The drugs were given at zero time. Broken curve, intraperitoneal injection of 40 mg/kg of compound I to mice; each point represents the 5-HT concentration of five pooled brains. Solid curve, intravenous injection of 40 mg/kg of compound I to rabbits; each point represents the 5-HT concentration of one whole brain. Dotted curve, intravenous injection of 5 mg/kg of reserpine to rabbits (*3*).

It has now been found that, besides reserpine, various synthetic derivatives of 1,2,3,4,6,7-hexahydrobenzo[a]quinolizines (*2*) also release 5-HT. In mice and rabbits, these compounds produce sedation without hypnosis. Among the derivatives examined, compound I (2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine)



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showed the most marked sedative and 5-HT-releasing activity (Fig. 1).

After injection of 40 mg of compound I per kilogram, there was an immediate decrease of the brain 5-HT, measured fluorimetrically (*3*), the minimum value being reached within half an hour. As the dose was reduced, the 5-HT decline became gradually smaller, but was still evident with as little as 5 mg of compound I per kilogram. The absolute decrease in 5-HT per gram of tissue was greater in the brain stem than it was in the rest of the brain. During a 4-hour period after injection of 40 mg/kg in rabbits, the colorimetrically determined excretion of 5-hydroxyindoleacetic acid (*4*), a major metabolite of 5-HT, showed an average significant increase of 200 percent as compared with a similar control period before injection ($p < 0.01$). In rabbits, pretreatment with isopropyl isonicotinic acid hydrazide had the same influence on the effect of compound I as on that of reserpine (*5*): compound I no longer caused sedation, but excitation, mydriasis, and piloerection; the brain 5-HT showed only a very slight decline.

In addition to these similarities between the action of compound I and reserpine on the brain 5-HT, there were, however, some differences. (i) To reach maximum depression of 5-HT in the brain, mice required 4 times and rabbits 10 times as much compound I as was required of reserpine. In mice, the LD_{50} of compound I was about 10 times higher than the LD_{50} of reserpine. (ii) After administration of reserpine, the 5-HT in the brain decreased to a minimum of 10 percent, whereas after administration of compound I, the 5-HT concentration was never less than 25 to 35 percent of the original value. Even with doses exceeding 40 mg of compound I per kilogram, no greater decrease of 5-HT could be produced. (iii) Within 10 to 24 hours after injection of compound I, the 5-HT content of the brain had returned to normal values, whereas, after administration of reserpine, complete 5-HT recovery took several days. The sedative action of compound I lasted 4 to 8 hours, that of reserpine 1 to 3 days.

The benzo-quinolizine derivatives are thus a second group of substances which, like the centrally acting *Rauwolfia* alkaloids, cause both sedation and 5-HT depression in the brain. Closer investigations with these compounds may lead to further explanation of the role of 5-HT in brain function and in the central action of certain drugs.

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13 May 1957

Nature of the Glucuronide in Direct-Reacting Bilirubin

Evidence from several laboratories (*1–3*) has established that direct-reacting bilirubin is a diglucuronide. Billing, Cole, and Lathe (*1*) have suggested that bilirubin may be conjugated with glucuronic acid through its carboxyl groups, since the glucuronide is readily hydrolyzed by dilute alkali. Schmid (*3*) has assumed that the glucuronidic linkages occur with the α,α′-hydroxy groups of bilirubin.

Carboxyl (acyl) glucuronides can be differentiated from other glucuronides by the capacity of the former to react with hydroxylamine to yield hydroxamic acids and glucuronic acid (*4*). Treatment of bilirubin diglucuronide with hydroxylamine, therefore, provides a means of determining the nature of the glucuronidic bonds (*5*).

Urines obtained from three patients with obstructive jaundice were the source of the direct-reacting bilirubin (*3*). In each instance, treatment of an aliquot of the urine with hydroxylamine (*p*H 7, room temperature, 30 minutes) resulted in the formation of an appreciable quantity of hydroxamate. When the urine