types of cattle twins which exhibit mosaicism for hemoglobin types can be established only on the basis of breeding tests.

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Protein-Bound Iodine in Serum of Rats Breathing 99 Percent Oxygen

Abstract. *Exposure of rats to 99 percent oxygen for 24 to 72 hours resulted in a significant fall in protein-bound iodine in serum. The fall was most prominent in the group treated for 72 hours and was not, however, associated with any detectable microscopic changes in thyroid structure.*

The influence of the thyroid gland on oxygen toxicity was first noted in 1937 by Campbell (*1*), who described increased survival in thyroidectomized rats exposed to 6 atmospheres of oxygen, while administration of exogenous thyroxine to normal rats enhanced toxicity. Similar findings (*2*) have been reported with regard to the elevated mortality and pulmonary damage induced by increased oxygen concentrations at atmospheric pressure. In addition, the measurable degree of protection afforded rodents by hypophysectomy can be counteracted by the administration of thyroid extract (*3*).

Despite these implications of thyroid participation, determinations of endogenous glandular activity and morphology have to our knowledge not been reported in previous investigations on animals exposed to oxygen-enriched environments. Our study, representing part of an overall investigation on the effects of pure oxygen systems programmed for use in spacecraft, was therefore designed to evaluate the protein-bound iodine (PBI) in serum and to study the thyroid histology in rodents inhaling 99 percent oxygen at 1 atmosphere pressure for periods of 24 to 72 hours.

Adult male albino rats (Wistar strain) weighing 240 ± 9 grams were used throughout the study and maintained on a diet of Purina rat chow (containing 1 part of iodine per million) and water as desired. Initially groups of 5, 6, and 20 rats were placed for 24, 48, and 72 hours, respectively, in a closed-system (*4*) environmental chamber in which they were exposed to 99 ± 0.5 percent oxygen at 22° to 26°C with a relative humidity of 41 to 57 percent. The experiment was then repeated with two groups of ten rats each, exposed for 24 and 48 hours, respectively, to conditions identical with those in the first series. The carbon dioxide was maintained at less than 0.2 percent by circulation of the chamber atmosphere through lithium hydroxide. Total pressure was kept at 25 mm-Hg above ambient to insure that all leaks should be "outboard." Immediately after removal from the chamber the rats were lightly anesthetized with diethyl ether, and blood was obtained from the abdominal aorta. A group of 24 rats maintained in individual cages in room air served as controls, and this group was treated identically. Serum PBI was determined by the method of Moran (*5*). Upon completion of aortic puncture, the thyroid was removed from each animal, fixed in formalin, sectioned, and stained with hematoxylin and eosin. The lungs were also examined for gross pathologic changes, and pleural fluid, if present, was withdrawn and its volume was measured.

The animals kept in oxygen for 48 hours or more appeared hyperpneic and lethargic (*6*), and at autopsy had characteristic diffuse areas of pulmonary consolidation. Ten of the rats died during the 72-hour-exposure period and were not used for blood or thyroid studies. Detectable pleural effusions amounting to 4 to 8 ml of serous fluid were noted in 80 percent of the rats surviving 72 hours in oxygen. Control animals in room air had no evidence of gross pulmonary changes or pleural effusion.

The results of the protein-bound iodine studies are summarized in Fig. 1. The data at 24 and 48 hours represent the combined results of two series of experiments. After only 24 hours, exposure to 99 percent oxygen, a highly significant ($P < .005$) fall in protein-bound iodine was noted. With longer exposure periods (48 and 72 hours), this decline became more striking and its statistical significance ($P < .001$) increased. Microscopic examination of the excised thyroids revealed cuboidal epithelium and normal amounts of colloid with no detectable differences between air controls and the rats inhaling oxygen. However, the exposure periods (no more than 72 hours) may have been insufficient to allow development of detectable structural changes.

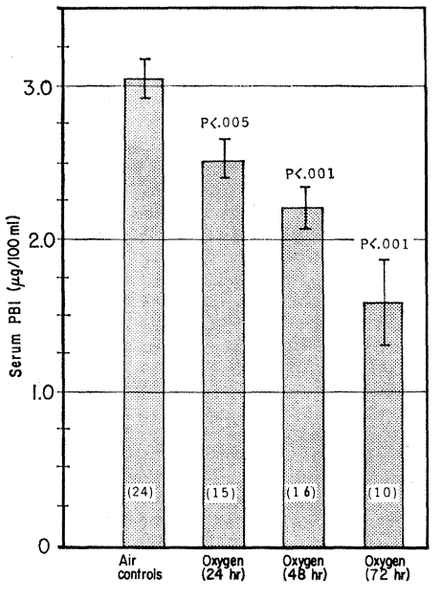

Fig. 1. Mean values for protein-bound iodine (PBI) in serum of control rats and rats exposed to 99 percent oxygen. The figures within each bar represent the number of rats in that group. Significance levels for differences from the control group are given above each bar. The vertical lines at the top of the bars represent the standard error (S.E. $\pm$ 1).

Diminution in protein-bound iodine may result from direct antithyroid action, suppression of thyrotropin secretion, or alteration in peripheral utilization and clearance of thyroid hormones. Reduction in thyroxine-binding capacity of plasma proteins may also be contributory. The mechanism responsible for the fall in hormone concentration accompanying oxygen inhalation remains to be determined.

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Behavior in Hydra: Inhibition of the Contraction Responses of Hydra pirardi

Abstract. Hydra pirardi *contracts spontaneously and in response to external stimuli of light and mechanical agitation. Inhibition of these contractions occurs when the animal feeds on* Artemia salina *or when reduced glutathione is present in the environment. Such inhibition demonstrates the control of one receptor-effector system by another in hydra.*

There has been renewed interest in the feeding reflex of hydra, stemming from the contention of Loomis (*1*) that reduced glutathione (GSH) specifically controls the feeding reflex of *Hydra littoralis*. Lenhoff (*2*) has systematically studied the role played by GSH in activating the feeding response in this species, and proposed a quantitative assay for the reflex based on the time of mouth opening of the animal. The uniqueness of GSH as an initiator of feeding in hydra has been questioned by Forrest (*3*) and Burnett *et al.* (*4*). Indeed, Forrest maintains not only that a large class of apparently unrelated substrates induce feeding in hydra, but also that the GSH-induced reaction is not a true feeding response of the animal.

In studies of the contraction responses of *Hydra pirardi* to stimuli of light and mechanical agitation, we observed that the characteristic contractions of the body column which occur in response to these stimuli (*5*) were inhibited while the animal was feeding on *Artemia salina*. The normal, spontaneous contractions of the animal were also temporarily eliminated during feeding. In this report we describe experiments on the inhibition of these contraction responses by feeding *H. pirardi* on *A. salina* and by adding reduced glutathione to the culture medium.

The animals we used in this study were from a clone of *H. pirardi*. The hydra were cultured by the method of Loomis and Lenhoff (*6*), except that distilled water was substituted for tap water in the culture medium. *Artemia salina* larvae were fed to the animals daily. The experimental animals were starved for 24 hours before being tested at 21°C. In experiments to determine the inhibition of the contraction response to light, two groups of ten hydra were exposed to a regime of 75 seconds of light followed by 75 seconds of dark (*7*). Each group of animals was placed in a petri dish containing 9 ml of culture water. Before each test, there was a control period of 15 minutes during which the numbers of animals in each dish contracting to five successive light periods were recorded. Substances were then pipetted into the dishes and the numbers of animals contracting to subsequent light periods were recorded. By this means, the effect of GSH and other chemicals of known concentrations were tested in addition to live *A. salina* or homogenates of *A. salina* (*3*).

Almost all animals contracted in response to the light stimulus in the control period before each test (Fig. 1). In marked contrast *A. salina*, both alive and in homogenate form, and $10^{-5}M$ GSH initially inhibited these contractions almost completely. The proportion of animals contracting to light gradually increased, however, until it became comparable to that of control animals. Thus, the original light response was restored after 50 to 65 minutes.

The behavior of the animal when exposed to light alone is markedly different from the response to light stimulation in the presence of *A. salina* or GSH. The usual contraction response to light alone consists of a successive series of partial body contractions culminating in the animal's forming a tight ball with contracted tentacles. The time between onset of the light and completed contraction of the animal (the reaction time) is a function of the intensity of the light and its spectral composition (*9*). The reaction time is inversely proportional to temperature (*10*). Under the present experimental conditions, the mean reaction time was 42.5 ± 10.2 seconds, based on 50 animals. In contrast to this light-induced contraction response *H. pirardi* did not exhibit the total contraction to light in the presence of *A. salina* or GSH. In this situation the tentacles writhed and twisted toward the mouth and the mouth itself opened widely (*11*). The movements of the animal were similar to those described by Ewer (*12*) and Loomis (*1*). After ingestion of *A. salina* circular contractions of the distal portion of the body column forcing the food down into the gastrovascular cavity of the animal, described by Forrest (see *3*) were frequently observed.

A second series of experiments was devised to demonstrate the inhibitory effect of *A. salina* and GSH on the contractional response to mechanical agitation. Two groups of five animals were placed in 48 ml of culture fluid in Stender preparation dishes. After the animals had attached themselves to the bottom of the dishes, they were shaken (*13*) for periods of 2 seconds every minute. During a control period before each test, the numbers of animals in the two groups contracting to the pulses of 2-second shaking were recorded for five trials. In experiments with live *A. salina* larvae, the hydra were provided with excess larvae and allowed to feed for 5 minutes. Then, the numbers of animals contracting to successive shaking periods were recorded. A similar procedure was used to study the effect of GSH. Figure 2 shows the results of inhibition of the contraction response to such mechanical agitation (*14*), both by live *A. salina* and by $10^{-5}M$ GSH. An effect similar to that with light stimulation was observed here, although inhibition was shorter, lasting for 25 to 30 minutes.

Live *Artemia salina* and GSH also inhibited the rhythmic spontaneous contractions of the animal. In a typical experiment, the numbers of total body contractions per 30-minute period for groups of ten animals in (i) culture fluid alone, (ii) live *A. salina*, and (iii) $10^{-5}M$ GSH were recorded. The mean number of completed contractions of hydra after providing excess numbers