were destroyed in our dogs. Brady and Nauta (*5*), and King (*6*) have pointed out that lesions of the septal region produce dramatic changes in emotionality. However, it appears from some reconstructions of the lesions described by them that the most striking hyperemotionality occurs when the destruction extends anteroventrally, including the regio genualis and parts of the subproreal area in addition.

Within the first postoperative days all animals with genual lesions showed increase in responses motivated by food reward. They appeared to expect food with both the positive and the negative CS, and took the food reward more vigorously than before. It is interesting to note that ablations of the medial precruciate (*7*) or pregenual (*8*) regions also impair the inhibitory food CR's. McCleary (*9*) has reported that subcallosal lesions "disrupt normal performance under circumstances requiring a frightened animal to inhibit responding." However, McCleary's subcallosal lesions included the regio genualis as well.

Recent evidence of Auleytner and Brutkowski (see *1*), indicating that dogs with prefrontal lobectomies temporarily lose their ability to inhibit the classical defensive CR's trained prior to operation, suggests that the medial forebrain areas in the dog (which seem to be homologous to the orbital areas in the monkey) are concerned with suppression of different kinds of motivated and affective responses mediated by hypothalamic mechanisms. Also parts of the amygdala appear to be involved in this system (*10*).

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Toxohormone-like Factor from Microorganisms with Impaired Respiration

Abstract. A substance that significantly depresses liver catalase values when injected into mice has been isolated from biochemical mutants of yeasts and staphylococci with impaired respiration. This is considered as an important argument in support of the Warburg hypothesis on the origin of cancer cells.

Biochemical studies by Warburg (*1*) in the last 30 years led him to the conclusion that transformation of a normal cell into a malignant one may be the result of irreversible damage to the cellular respiratory mechanism. This respiration deficiency, typical of cancer cells, can be induced in microorganisms. If the Warburg hypothesis is true, microbial mutants with impaired respiration would be the "equivalents," among microorganisms, of cancer cells (see *2*), and their biochemical characters would agree with those of malignant cells.

Such a biochemical resemblance seems to be confirmed by studies on some of the most important biochemical features of respiration-deficient microorganisms (*2, 3*).

As a continuation of that line of research, we investigated whether microorganisms with impaired respiration produce certain toxic metabolites like those produced by cancer cells: substances such as the toxohormone of Nakahara and Fukuoka (*4*), a factor which depresses liver catalase.

The production of toxohormone-like substances by respiration-deficient mutants of microorganisms would indicate that these mutants are "equivalents" of cancer cells. Such production would be an important argument in support of the Warburg hypothesis on the origin of cancer cells.

Five different yeast mutants with impaired respiration and two staphylococci mutants were used in the experiments. The yeast mutants were obtained from a parent strain of *Saccharomyces cerevisiae* by treatments with Trypaflavin (*2*) (T_1 and T_4 mutants), manganese (*5*) (Mn_2 and Mn_3 mutants), and methyl violet (*6*) (MV_2 mutant). The mutants were selected by the tetrazolium overlay technique (*7*). They had the following characteristics: Q_{O_2} (N) between 0 and 50; inability to grow on lactate agar; uncolored colonies after overlay with 2,3,5-triphenyltetrazolium chloride; and no alkali production in an acetate medium (*8*).

The staphylococci mutants were obtained from a parent strain of *Staphylococcus aureus* by irradiation with ultraviolet (UV_5 and UV_6 mutants). They had a Q_{O_2} about 45 percent of that of the parent strain, and their respiration was only slightly depressed by 0.02*M* sodium cyanide.

To obtain cell mass, mutants and parent strains were grown for 72 hours in 10-liter fermenters with aeration and stirring. Yeasts were grown in beer wort at 30°C. Staphylococci were grown in peptone-meat extract saline broth at 37°C. Cells were recovered by centrifugation, dried with acetone, and powdered.

One hypothetical toxohormone-like fraction (TH) was obtained from acetone-dried powder of each strain by the Yunoki-Griffin technique (*9*) for preparation of crude toxohormone from malignant tissues; about 1.50 g were obtained from 50 g of acetone-dried powder.

Activity was assayed by injecting preparations of the toxohormone-like fractions into mice and measuring the liver catalase activity. Toxohormone-like fractions were dissolved in distilled water in a concentration of 50 mg/ml. For the assay 0.5 ml of the solution was injected into the peritoneal cavity. After a single injection the mice were deprived of food for 24 hours and then killed. Liver catalase activity was determined by the technique of Bonnichsen *et al.* (*10*) and expressed in terms of the reaction rate per minute, divided by the dry weight of the preparation in grams (*11*).

Table 1 shows the liver catalase activity for mice injected with 25 mg

Table 1. Liver catalase values in mice injected with toxohormone-like fractions (TH) from *Saccharomyces cerevisiae* and *Staphylococcus aureus* and from their mutants with impaired respiration. The liver catalase activity, Kat. f., is the reaction rate per minute, divided by the dry weight of the preparation in grams.

Treatments	No. of mice	Liver catalase activity (Kat. f.)
None; mice fasted for 24 hours	110	75.3 ± 1.7
25 mg of TH from:		
S. cerevisiae		
Parent strain	20	70.0 ± 7.2
Mutant T_1	10	43.9 ± 10.1*
Mutant T_4	10	38.6 ± 5.7*
Mutant Mn_2	10	49.4 ± 3.1*
Mutant Mn_3	10	44.4 ± 3.9*
Mutant MV_2	10	35.6 ± 9.9*
S. aureus		
Parent strain	20	69.8 ± 2.7
Mutant UV_5	10	53.1 ± 3.0*
Mutant UV_6	10	49.8 ± 3.2*

* Significantly different from untreated mice ($P < .01$).

of different toxohormone-like fractions and for untreated mice.

Toxohormone-like fractions from mutants significantly reduced liver catalase values when injected into mice, whereas catalase values were not significantly altered in mice that were injected with toxohormone-like fractions from parent strains of the microorganisms.

From these results it can be assumed that respiration-deficient mutants of microorganisms, at least in some species, produce a toxohormone-like factor or catalase-depressing factor which has the same effect as substances produced by cancer cells.

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Species Differentiation in Fish by Electrophoretic Analysis of Skeletal Muscle Proteins

Abstract. Extracts of skeletal muscle protein from ten species of the fresh-water sunfish family were examined by the electrophoretic technique, and the resulting patterns showed characteristic specificity in each instance. Usually three major components of greater mobility were accompanied by slower moving minor components. No uniformity of mobility or of proportion of protein was evident. Thus patterns that "fingerprint" the species are evident.

The moving-boundary method of electrophoresis was adapted by Connell (*1*) to the analysis of skeletal-muscle proteins of fish. In analyses of 20 species he found a very characteristic electrophoretic pattern for each species, as well as considerable differences in component mobilities and composition. Much additional information about proteins of fish has been reviewed by Hamoir (*2*). We have extended the same method of skeletal-muscle-protein analysis to several families, genera, and species of fish native to the Great Lakes region in Michigan. In this work we have sought to ascertain whether this procedure might provide another physicochemical means of distinguishing taxonomic differences.

The fish in this study, with the exception of the white crappie (*Pomoxis annularis*), were captured during summer months from lakes and streams in the vicinity of the W. K. Kellogg Gull Lake Biological Laboratory near Battle Creek, Michigan (*3*). Shortly after capture, specimens were placed in a deep-freeze unit and stored until the analysis was to be made. To prepare the sample for electrophoresis, each specimen was thawed sufficiently to permit easy removal of the skin, and with the aid of a scalpel about 20 g of skeletal muscle was removed from a region just ventral to the dorsal fin. The muscle was thoroughly mixed in a Waring blender, at room temperature, with 20 ml of buffer composed of 0.0156*M* Na_2HPO_4 and 0.0035*M* KH_2PO_4 at *p*H 7.5 and ionic strength of 0.10. The mince was allowed to settle, and a portion of the supernatant was decanted, placed in a 15-ml centrifuge tube, and clarified for 15 minutes at 1500 rev/min in an International Clinical Model centrifuge. The sample was then dialyzed for a 12-hour period against the buffer described, and electrophoresis was carried out in the Perkin-Elmer model 38 Tiselius apparatus. All runs were carried out at ice-water temperature for 6300 seconds, under a potential gradient of 8.5 volt/cm. The protein concentration was maintained between 1 and 1.5 percent, and a 2-ml conductivity cell (constant = 0.4893) was used to measure the resistance of the dialyzed protein samples at ice-water temperature.

The electrophoretic patterns obtained from ten species representing six genera of the family Centrarchidae, the fresh-water sunfish family, are shown in Fig. 1. These patterns show a great diversity in number and location of components as well as nonenantiography between the ascending and the descending boundary curves. Electrophoretic mobilities of the discernible peaks averaged from the ascending and descending patterns clearly showed further differentiation between the species. While some minor components may be barely distinguishable, the electrophoretograms are typical for the species considered, and runs made on other individuals of the same species always give the same pattern.

There is an overall similarity in the electrophoretograms of Fig. 1 in that several major components (usually three) are present in the middle or leading range of the patterns. Each pattern is made up of a set of component mobilities different from those of any other pattern. This variation, which according to Hamoir (*2*) characterizes muscle extract from other species of fish, is very evident for the centrarchid species studied in this work. Although these protein extracts consist principally of enzymes that have the same functions in muscle glycolysis, the electrophoretic specificity of the extracts is pronounced. The patterns are quite similar to those of marine species, as given by Connell (*1*), with respect to the major components but differ in kind and proportion for the minor ones.

The minor components in the patterns of Fig. 1 seem to exist in the more slowly moving region and appear too ill-defined to warrant any special designation. Only the patterns for the

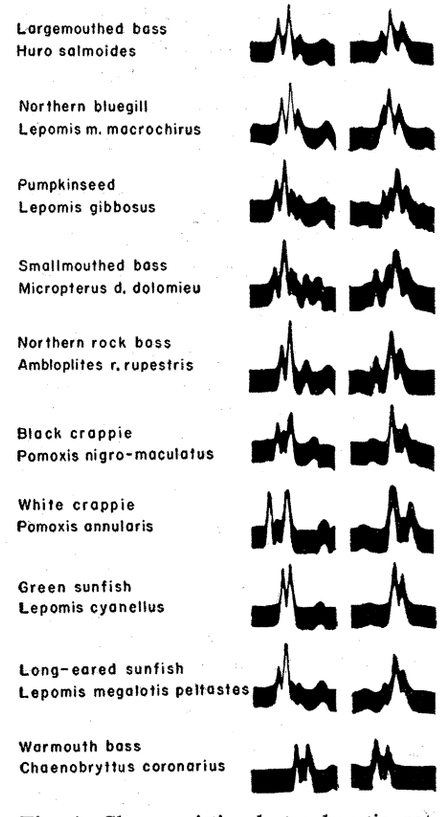

Fig. 1. Characteristic electrophoretic patterns of skeletal muscle proteins from ten species of fresh-water sunfish (family Centrarchidae). Patterns at left, ascending; at right, descending.