RESPIRATION OF THE TISSUES OF SOME INVERTEBRATES AND ITS INHIBITION BY CYANIDE*

By W. A. ROBBIE

(From the Departments of Ophthalmology and Physiology, College of Medicine, State University of Iowa, Iowa City)

(Received for publication, March 7, 1949)

Present theories of the mechanisms of cell respiration have been developed primarily from investigations on only a few types of living material: yeast cells, sea urchin eggs, pigeon breast muscle, and mammalian tissues. The concepts derived from these studies may not be applicable to all animal tissues: not only may certain details in the picture be lacking, but the extent to which the conventional cytochrome-cytochrome oxidase scheme participates is in many cases as yet undefined. Although Keilin (1) more than twenty years ago confirmed the widespread occurrence of cytochrome in living cells, there are still many gaps in our knowledge of the distribution of this compound among the invertebrates. Also, the mere presence of cytochrome c in a cell gives no indication of the extent to which the cell is actually dependent upon the cytochrome system for its normal activity. The present study was undertaken to obtain comparative cell respiration measurements of certain representative invertebrate tissues and to determine how much of this oxygen consumption is dependent upon cyanide-sensitive mechanisms.

Experimental Technique

This investigation was made at the Bermuda Biological Station for Research during the fall and winter of 1947. Respiration measurements were obtained with a Warburg manometric outfit, using standard and micro flasks of about 17 cc. and 6 cc. volumes respectively. The rate of shaking was kept at 120 cycles per minute, with variation of the amplitude according to the nature of the material. The oxygen consumption was determined with the tissues in filtered sea water, with a 10 per cent KOH solution or a 10 per cent $Ca(OH)_2$ suspension in the center wells to absorb CO_2. Since the temperature of the water from which the animals were collected remained within a range of 27 to 17°C. during the course of the observations it was possible to make the observations at the relatively high level of 25°C.[1]

Respiratory measurements on most of the tissues could be made without slicing

*Aided by a grant from The John and Mary R. Markle Foundation.

[1]During the summer it was difficult to keep certain animals in the laboratory for more than a few hours, but in early October when the temperature of the sea water supply dropped below 25°C. it was found that most of the specimens could be maintained in good condition for several days or even weeks.

the material since the structures themselves were within the limiting thickness. However, slices were made with a razor blade through the sponges and through the calcareous material along the sides of the gorgonian rods. It was possible to use rather thick slices of the sponges since their porous nature permitted rapid circulation of sea water. The branches of the purple sea fan were ground in a porcelain mortar, and the cellular suspension was decanted from the heavy skeletal debris. Barnacles were removed from their calcareous outer shells with as little injury as possible and measured without further treatment. The inert outer tunic was removed from the small tunicates, and with the large, simple tunicates, only the pharynx was studied.

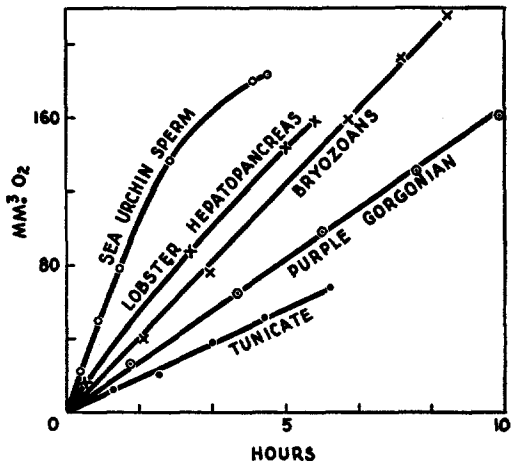

FIG. 1. Typical oxygen consumption curves for various invertebrate tissue preparations in Warburg manometer flasks in sea water. The bryozoans were intact; the tunicate had the inert tunic removed; lateral slices were made from along the sides of the gorgonian rod; the filamentous lobster hepatopancreas bundles were separated from each other.

Oxygen consumption determinations are expressed in the tables as Q_{O_2} values (mm.^{3}O$_2$/mg. dry weight/hr.). It was necessary to correct the gross dry weight measurements of those tissues which contained inert skeletal material, such as the sponges, coral animals, and barnacles. Samples of these tissues were dried and weighed, treated with trichloroacetic acid to fix the proteins, with 25 per cent HCl, washed, centrifuged, then redried and weighed. This eliminated the $CaCO_3$ and the soluble salts. The trichloroacetic acid was driven off during the drying period at 100°C. Further treatment with boiling 10 per cent KOH to dissolve the protein, washing, centrifuging, and a final weighing then gave the amount of inert siliceous or chitinous material. From these weights it was possible to compute the original dry weight of the tissue minus the skeletal material.

It is difficult to determine whether measurement of the oxygen consumption of a piece of isolated tissue is truly representative of the respiration of the tissue in its

normal environment. The course of oxygen uptake, however, may at least indicate whether there is a progressive change in the chemical systems in the tissue which are responsible for respiration. Fig. 1 shows typical oxygen consumption curves for several different types of tissue. Some specimens, such as the bryozoans and gorgonians, could be run for as long as 10 hours without decrease in rate. The bryozoans were probably uninjured since every animal is protected by enclosure in a small, transparent shell, partially open on one side to allow circulation of sea water. Each polyp in the gorgonian slices must have been cut, but the injury was apparently localized and did not affect the intact adjoining cells. Most tissues could be measured for at least a 2 to 3 hour period without appreciable falling-off in respiration. Certain types, such as the lobster tissues and some of the sponges, showed a continuously decreasing rate but this probably affected the measurement only slightly during the first

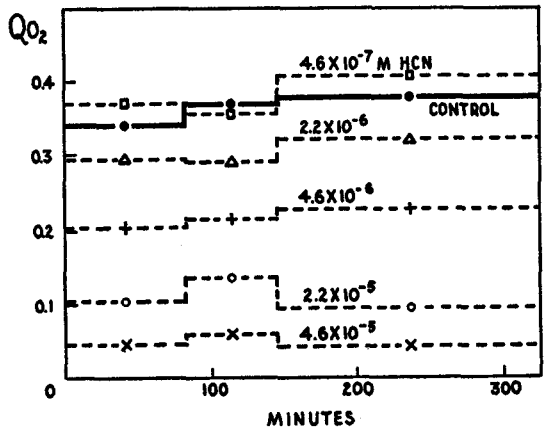

FIG. 2. Respiration of the epithelial eye covering or "cornea" of the squid in various concentrations of cyanide in sea water. The HCN tensions were maintained at constant levels by $Ca(CN)_2$ - $Ca(OH)_2$ center well mixtures.

hour or two. The only materials which were found to show an increase in the rate of oxygen consumption upon shaking were the sea urchin eggs (Fig. 6). This increase was probably due to development in the fertilized eggs, and to instability of the unfertilized ones.

In order to maintain constant concentrations of HCN in the sea water in the flasks, $Ca(CN)_2$–$Ca(OH)_2$ solutions were used in the center wells (2). These "balanced" center well solutions absorb CO_2 without altering the tension of HCN in the air space in the flasks.[2] It was found that HCN equilibrium between the center well mixture and the sea water is attained so rapidly that it is unnecessary to add cyanide directly to the sea water itself. For example, in the experiment represented in Fig. 2, ma-

[2]Although sea water becomes alkaline when its CO_2 is absorbed by the center well solution, the respiratory CO_2 was sufficient to prevent extensive shift in pH.

nometer readings were started 20 minutes after cyanide had been added to the filter paper in the center wells. The inhibition over the subsequent 5 hour period was constant, indicating that approximate HCN equilibrium was reached by the time the first reading was taken. This technique is convenient and it is also desirable because of the fact that nothing is added to the fluid containing the experimental material except the HCN gas from the center well mixture. A biological check of the HCN equilibrium levels of the center well solutions has shown that, even at 1×10^{-6} M, the concentration of HCN in the sea water is still at the theoretically correct level (3).

RESULTS

(*a*) *Respiratory Rate.*—The QO_2 values of the tissues studied are listed in Table I. Where there are ten or more determinations standard deviations are given. The figures range from extremes of 0.02 for the squid lens to 26 for sea urchin sperm, but the other values lie within a range of 0.4 to 3.0.

(*b*) *Respiration in Cyanide.*—Inhibition of respiration of tissues in various cyanide solutions is indicated by the values in Table II. Sea urchin sperm, squid gills, and lobster nerve and muscle are almost completely inhibited by low concentrations of cyanide, but many of the animals show only a partial sensitivity. The oxygen consumption of three tissues, the jellyfish, *Cassiopea*, the branchial tree of the sea cucumber, and the tunicates, was entirely unaffected by even 0.01 M HCN.

The constant rates of respiration in cyanide demonstrated by the curves in Fig. 2 are typical, although with a few kinds of animals high concentrations of HCN resulted in a gradual falling-off of oxygen consumption. This deviation from a constant rate was not usually evident until after more than 2 hours' exposure.

(*c*) *Concentration of HCN Necessary for 50 Per Cent Inhibition.*—Fig. 3 illustrates the typical spread of points obtained when observed oxygen uptake is plotted against the concentration of cyanide. Fig. 4 shows concentration-inhibition curves for a number of representative tissues. The concentration of cyanide necessary to cause 50 per cent inhibition of the sensitive portion of the respiration may range from 10^{-6} M (sea urchin sperm) to 10^{-3} M (sea fan). This widespread variation is indicated by the values in Table III for the equilibrium constant, K, which denotes the concentration of cyanide that produces 50 per cent inhibition of the sensitive portion of the respiration.

(*d*) *The Slope of the Inhibition Curve.*—A comparison of the curves shown in Fig. 4 is facilitated by applying the law of mass action to the data. The curves in Fig. 5 are derived by plotting the logarithm of the ratio of the inhibited to the uninhibited respiration against the log of the concentration of the inhibitor (4). (Since it is only the cyanide-sensitive part of the respiration which is being analyzed the portion unaffected by 10^{-2} M HCN was not considered.) Table III gives the slopes of the straight portions of the

TABLE I

Oxygen Consumption of Tissues of Bermuda Invertebrates

Animal	Tissue	No. of determinations	Q_{O_2} Mean	Q_{O_2} Range
Sponges				
(*Tedania ignis*)	Slices	6	−2.9	−2.5 to −3.7
(*Ircinia fasciculata*)	"	5	−1.6	−1.2 to −2.1
(*Lissodendoryx isodictyalis*)	"	4	−1.4	−1.1 to −1.6
(*Axinella rosacea*)	"	2	−0.7	−0.6 to −0.8
(*Cinachyra cavernosa*)	"	3	−0.6	−0.5 to −0.6
(*Dysidea crawshayi*)	"	2	−0.6	−0.6 to −0.7
(*Terpios fugax*)	"	1	−0.6	
(*Geodia gibberosa*)	"	1	−0.6	
(*Tethya aurantia*)	"	1	−0.5	
(*Spheciospongia sp.*)	"	1	−0.4	
Coelenterates				
Purple gorgonian (*Plexaura flexuosa*)	Slices	13	−3.0 ±.66	−2.4 to −4.5
Purple sea fan (*Gorgonia flabellum*)	Suspension	2	−2.2	−2.1 to −2.3
Portuguese man-of-war (*Physalia pelagica*)	Tentacles	3	−1.7	−1.1 to −2.2
Sea anemone (*Condylactis gigantea*)	"	3	−0.8	−0.7 to −0.9
Jellyfish (*Pelagia cyanella*)	Umbrella	2	−0.8	−0.8 to −0.9
(*Cassiopea frondosa*)	"	18	−0.7 ±.17	−0.3 to −1.0
(*Cassiopea frondosa*)	Tentacles	10	−0.6 ±.16	−0.4 to −1.0
Bryozoans				
Species of *Ectoprocta*	Entire	6	−2.1	−1.9 to −2.5
Echinoderms				
Sea urchin (*Tripneustes esculentus*)	Sperm	3	−26	−18 to −41
" "	Eggs, (fertilized)	3	−0.6	−0.6 to −0.7
" "	Eggs, (unfertilized)	6	−0.1	−0.08 to −0.13
Sea cucumber (*Stichopus möbii*)	Intestine	12	−0.7 ±.10	−0.6 to −1.0
" "	Branchial tree	11	−0.6 ±.07	−0.5 to −0.7
Mollusks				
Squid (*Loligo pealei*)	Gills	5	−1.8	−1.4 to −2.3
" "	Retina	3	−1.1	−0.9 to −1.4
" "	"Cornea"	4	−0.4	−0.4 to −0.5
" "	Lens	2	−0.02	−0.02 to −0.03
Black oyster (*Pedalion alata*)	Gills	6	−1.3	−0.9 to −2.7

TABLE I—*Concluded*

Animal	Tissue	No. of determinations	Q_{O_2}	
			Mean	Range
Crustaceans				
Goose neck barnacle				
(—)*.....	Entire	2	−3.0	−2.2 to −3.9
Lobster				
(*Panulirus argus*).....	Hepatopancreas	4	−3.0	−2.3 to −3.8
" "	Leg nerve	5	−1.1	−0.9 to −1.4
" "	Leg muscle	2	−1.0	−0.7 to −1.2
Tunicates				
Social tunicate				
(—).....	Entire animal except tunic	7	−1.3	−1.0 to −1.5
Simple tunicate				
(—).....	Pharynx	10	−1.1 ±.19	−0.8 to −1.4
Fish				
Red snapper				
(*Lutianus vivanus*).....	Retina	2	−2.1	−2.0 to −2.1

Q_{O_2} values are determined on a corrected dry weight basis (see text). Standard deviations are listed when there are ten or more determinations.

* Dashes indicate that scientific name was not determined.

curves. All the values approximate 1, except those for the relatively insensitive coelenterates. However, the data given in Table II but not plotted in Fig. 5 indicate that two of the sponges, the sea cucumber intestine, and the oyster gills would also give slopes of a value different than 1. The curve for the sea fan suspension may not represent a normal respiratory system, since the tissues were crushed and this disruption of the cells may have permitted spontaneous oxidation of endogenous substrate.

(*e*) *Experiments with Cassiopea.*—The jellyfish, *Cassiopea*, is one of the three forms listed in Table II whose respiration was completely unaffected by even high concentrations of HCN. Most of the oxygen consumption of this animal takes place in the cellular surface layer of either the tentacles or the dorsal or ventral coverings. The surface of the subumbrella is easily removed by cutting wedge-shaped pieces through the flat body and slicing off a millimeter or so with a razor blade. The denuded jelly layer had an oxygen consumption, on a dry weight basis, of only about one twenty-fifth the rate of the surface tissue. Results of experiments performed on the active surface cells are recorded in Table IV. Neither HCN nor 10^{-1}M NaN_3 produced inhibition of respiration within a 2 hour period, although the same concentration of the latter agent caused an 87 per cent depression of the oxygen con-

TABLE II

Respiration of Tissues of Bermuda Marine Animals in Cyanide

Animal	Tissue	No. of determinations	O_2 consumption in HCN (Per cent control)				K
			10^{-2} M	10^{-3} M	10^{-4} M	10^{-5} M	
Sponges							
(*Tedania ignis*)	Slices	18	27	30	34	56	$10^{-5.2}$
(*Ircinia fasciculata*)	"	18	21	24	28	56	$10^{-5.6}$
(*Dysidea crawshayi*)	"	12	26	27	30	48	$10^{-5.4}$
(*Axinella rosacea*)	"	6	47	59	72	84	$10^{-4.2}$
(*Cinachyra cavernosa*)	"	6	29	49	63	80	$10^{-4.0}$
(*Haliclona viridis*)	"	18	28	31	37	53	$10^{-5.4}$
(*Chondrilla nucula*)	"	18	26	27	30	59	$10^{-5.1}$
(*Spirastrella coccinea*)	"	1		77			
(*Lissodendoryx isodictyalis*)	"	4		31			
(*Tethya aurantia*)	"	1		59			
(*Terpios fugax*)	"	1		45			
(*Spheciospongia sp.*)	"	1		68			
(*Geodia gibberosa*)	"	1		43			
(*Leuconia barbata*)	"	1		19			
Coelenterates							
Sea anemone (*Condylactis gigantea*)	Tentacles	18	37	50	63	79	$10^{-4.3}$
Purple gorgonian (*Plexaura flexuosa*)	Slices	22	39	57	81	97	$10^{-3.5}$
Brown gorgonian (*Pseudoplexaura crassa*)	"	22	37	56	75	96	$10^{-3.7}$
Purple sea fan (*Gorgonia flabellum*)	Suspension	23	32	64	99	100	$10^{-3.0}$
Jellyfish (*Cassiopea frondosa*)	Subumbrella	25	100	100	100	100	
Portuguese man-of-war (*Physalia cyanella*)	Tentacles	12	12	16	46	87	$10^{-4.2}$
Bryozoans							
Species of *Ectoprocta* (—)	Entire	12	20	21	29	66	$10^{-4.9}$
Echinoderms							
Sea cucumber (*Stichopus möbii*)	Intestine	13	36	42	58	80	$10^{-4.4}$
" "	Respiratory tree	16	100	100	100	100	
Sea urchin (*Tripneustes esculentus*)	Eggs, (unfertilized)	20	27	27	37	72	$10^{-4.8}$
" "	Eggs, (fertilized)	16	12	12	13	25	$10^{-5.6}$
" "	Sperm	15	3	3	4	7	$10^{-6.1}$

TABLE II—*Concluded*

Animal	Tissue	No. of determinations	O_2 consumption in HCN (Per cent control)				K
			10^{-2} M	10^{-3} M	10^{-4} M	10^{-5} M	
Mollusks							
Black oyster							
(*Pedalion alata*)	Gills	12	24	30	38	46	$10^{-5.8}$
" "	Mantle	12	20	20	21	33	$10^{-6.7}$
Arca							
(*Arca noae*)	Gills	6	67	73	78	84	$10^{-6.0}$
Squid							
(*Loligo pealei*)	Gills	17	6	6	10	52	$10^{-5.0}$
" "	Retina	15	22	24	28	56	$10^{-5.0}$
" "	Lens	10	18	20	27	61	$10^{-5.0}$
" "	"Cornea"	11	20	20	20	48	$10^{-5.2}$
Crustaceans							
Lobster							
(*Panulirus argus*)	Leg nerve	12	3	4	6	19	$10^{-5.3}$
" "	Leg muscle	6	4	5	14	30	$10^{-5.6}$
" "	Hepatopancreas	12	20	21	30	78	$10^{-4.7}$
Goose neck barnacle							
(—)	Entire	12	12	16	24	56	$10^{-5.0}$
Tunicates							
Large simple tunicate							
(—)	Pharynx	15	100	100	100	100	
Small social tunicate							
(—)	Entire animal except tunic	12	100	100	100	100	
Fish							
Red snapper							
(*Lutianus vivanus*)	Retina	9	7	9	22	84	$10^{-4.5}$

The K values in the last column represent the HCN concentration which produces 50 per cent inhibition of the cyanide-sensitive portion of the respiration.

sumption of squid gills. After 3 hours in 10^{-2}M HCN, the jellyfish subumbrellar tissue began to deteriorate and the oxygen consumption declined, but the intact animals could be kept in 10^{-3}M HCN in sea water for at least 48 hours without apparent injury. The cyanide-sea water for this experiment was changed frequently to maintain adequate oxygen tension and also to assure that the tissues did not deplete the cyanide by some process of detoxication. Survival of muscular movement and active nerve conduction was shown by the regular periodic contractions of the animal.

After the *Cassiopea* subumbrellar tissue had been warmed to 50°C. for 5 minutes, the subsequent respiration was about one-fifth normal, and when

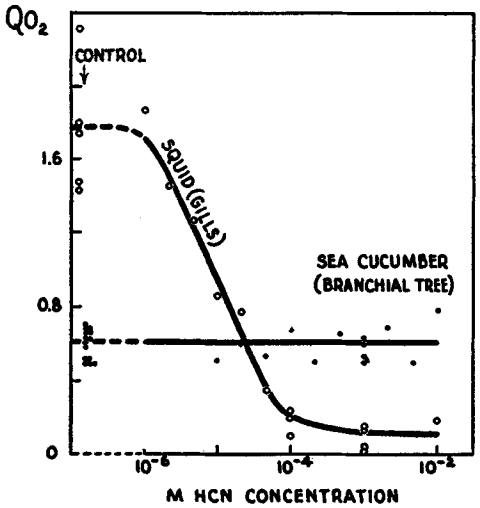

FIG. 3. Representative cyanide inhibition data for a sensitive tissue (squid gills), and one which is unaffected by HCN (sea cucumber branchial tree).

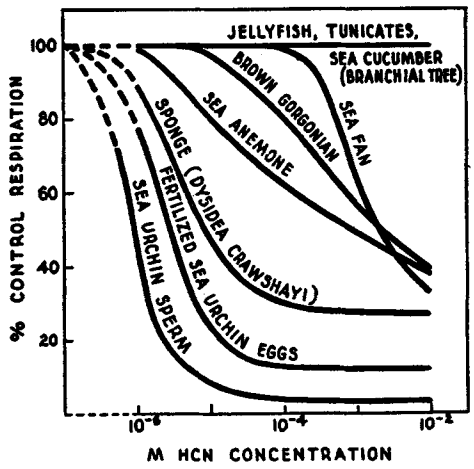

FIG. 4. Variation in oxygen consumption with concentration of cyanide for nine types of marine invertebrate material. Species names are listed in Table II.

heated to 65°C. there was a further reduction to one-fifteenth that of the control, indicating that a heat-sensitive enzyme system is involved. Drying the tissue for 7 hours at only slightly elevated temperature also resulted in

almost complete loss of oxygen uptake. Crushing the cells in a glass tissue grinder lowered the respiration rate about 50 per cent.

TABLE III

Slope Values for the Curves Shown in Fig. 5

Curve	Tissue	Slope
1	Red snapper retina	1.22
2	Rat spleen (5)	1.26
3	Rat retina (6)	1.03
4	Rabbit lens (7)	1.05
5	Sea urchin sperm	0.88
6	Fertilized sea urchin eggs	1.16
7	Squid gills	0.98
8	Sponge (*Dysidea crawshayi*)	1.08
9	Sea anemone tentacles	0.46
10	Brown gorgonian	0.76
11	Sea fan	1.93 and 1.31

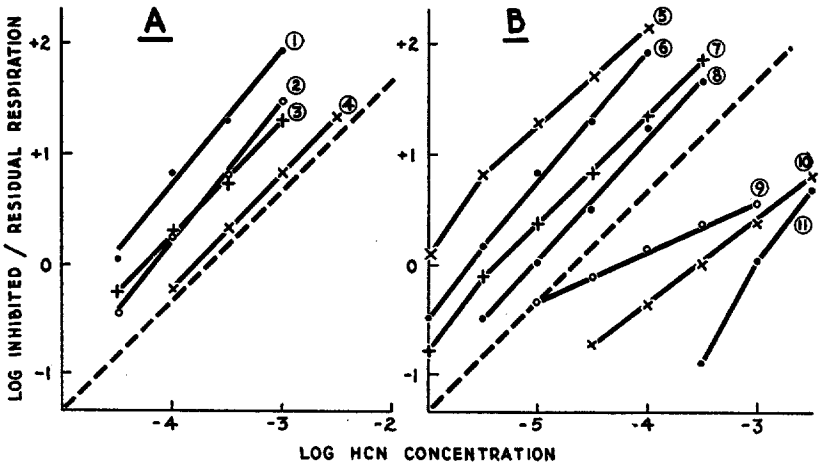

FIG. 5. Log ratio inhibited respiration/residual respiration against log cyanide concentration for vertebrate tissues (A), and invertebrate tissues represented in Fig. 4 (B). Types of tissues are listed in Table III. All values are corrected by subtracting the cyanide-resistant respiration at 10^{-2}M HCN.

Exposure to H_2S-saturated sea water for 10 minutes resulted in a considerable rise in oxygen consumption, but this may have been due to oxidation of the adsorbed sulfur during the subsequent measurement period. Intracellular pH factors are important in determining whether the sulfide will combine effectively with heavy metal, and the negative results obtained do

TABLE IV

Oxygen Consumption of the Subumbrellar Surface of the Jellyfish, Cassiopea frondosa, with Various Experimental Treatments

Treatment	Q_{O_2}
Control; under surface of umbrella	−0.70
Control; jelly layer	−0.025
Unaffected	
HCN, 10^{-2}M, 2 hrs.	−0.75
HCN, 10^{-3}M, 45 hrs. (unchanged)	——
NaN_3, 10^{-1}M	−0.61
NaF, 10^{-2}M	−0.65
5 min. at 35–37°C	−0.77
Inhibited	
5 min. at 50–51°C	−0.15
5 min. at 65–66°C	−0.05
Dried 7 hrs., at about 35°C	−0.06
Ground in glass tissue grinder	−0.33
Stimulated	
H_2S-saturated sea water, 10 min	−2.9
Methylene blue, 10^{-3}M	−2.0
Toluylene blue, 10^{-3}M	−2.7

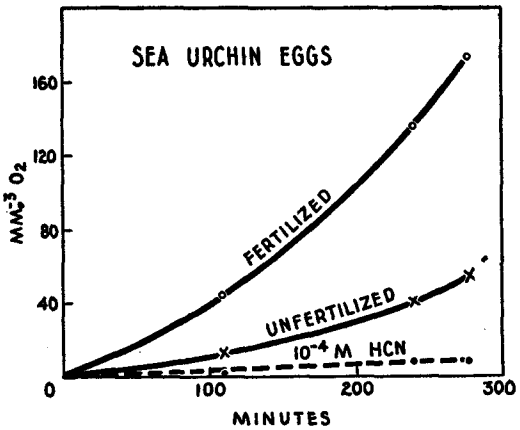

FIG. 6. Oxygen consumption of a suspension of sea urchin eggs (*Tripneustes esculentus*) before and after fertilization, and in 10^{-4}M HCN. The curves for both fertilized and unfertilized eggs in cyanide are identical.

not necessarily mean that no heavy metal is active. Methylene blue and toluylene blue were also tried. They caused some acceleration of oxygen uptake.

(*f*) *The Effect of Cyanide on Sea Urchin Eggs.*—The oxygen uptake of the eggs of the white sea urchin, *Tripneustes esculentus*, is represented by the curves in Fig. 6. There is a three- to fivefold stimulation of respiration on fertilization, which is comparable to that of *Arbacia* and other sea urchins. The effect of various concentrations of cyanide on the oxygen consumption of the eggs is listed in Table II. Fig. 6 shows that 10^{-4}M HCN almost completely inhibits the oxygen consumption of both unfertilized and fertilized eggs and that the residual respiration of both in cyanide is identical. Complete inhibition of cell division occurs at a concentration of slightly less than 10^{-5}M HCN. The respiration of the fertilized egg in this solution is reduced to about 40 per cent of the control level.

DISCUSSION

A comparison of the respiration of tissues from marine invertebrates at 25°C. with that of vertebrate tissues at the same temperature shows that in some cases the former may approximate the latter (Fig. 7). Although certain of the lower animals are metabolically sluggish there are others that can apparently function as actively as the higher forms if the temperature level is comparable. The cyanide sensitivity studies show that heavy metal systems participate in the cellular respiration of most primitive forms. If such animals as the bryozoans have maintained as much physiologic constancy during geologic history as they have morphologic uniformity, then it may be said speculatively at least that the use of heavy metal electron transfer systems in cell respiration may have been one of the first steps in the evolutionary development of living matter.

A quantitative study of the inhibition of cellular respiration by cyanide can provide three types of information: (1) the magnitudes of the cyanide-sensitive and insensitive portions; (2) the concentration of HCN required to produce 50 per cent inhibition (the equilibrium constant K value); and (3) the slope of the inhibition curve.

Although depression of oxygen consumption by low concentrations of cyanide is indicative of heavy metal catalysis, it is not conclusive evidence of cytochrome oxidase activity. Cyanide may inactivate copper and other heavy metals as well as iron, and possibly other mechanisms than the cytochrome system are active in certain animals. The cyanide experiments are valuable because this agent penetrates uninjured cells and indicates how much of the normal respiration is mediated by sensitive systems.

The cyanide-resistant respiration is plotted in Fig. 7 in actual QO_2 values rather than as percentage inhibition, since Commoner (10) has shown that, for several tissues at least, the cyanide-resistant portion is constant but the magnitude of the sensitive portion may increase greatly upon the addition of substrate. It may be noted in this connection that the vertebrate tissues

represented in Fig. 7 were in saline solution containing glucose, whereas the invertebrates had endogenous substrate only.

Several hypotheses have been suggested to account for the cyanide-insensitive portion of cellular respiration: (*a*) Gourévitch (11) found that those tissues which have a high riboflavin content are most insensitive, perhaps because flavoprotein can be directly oxidized by molecular oxygen. (*b*) Certain stages of fat and protein oxidation are also independent of the cytochrome system (10), and are therefore not sensitive to cyanide. (*c*) Since cytochromes

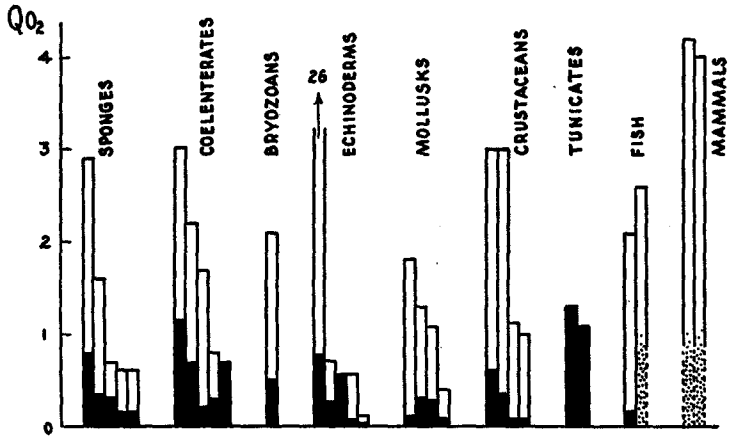

FIG. 7. A graphic comparison of the respiration and cyanide sensitivity of invertebrate and vertebrate tissues (25°C.). The solid portions of the columns indicate residual respiration in 0.01 M HCN. The last three columns represent oxygen consumption of bass brain (8), rat liver (9), and rat brain (8) respectively. The lower parts of these columns are left indeterminate by dotting since measurements of the cyanide sensitivities at this temperature are not available. Tissues represented by the other columns may be identified from the values in Tables I and II.

a and b do not combine with cyanide a certain amount of oxidation may proceed even in the presence of the inhibitor (12). (*d*) Another explanation is that although the addition of cyanide to cytochrome oxidase lowers the oxidation-reduction potential, the resulting compound may still be capable of oxidizing flavoprotein (13).

Complete insensitivity of the respiration of a living tissue to cyanide has seldom been observed. *Paramecium caudatum* is the classic example of a "cyanide-insensitive" organism, but Pace (14) has recently demonstrated that it is only the starved animals that are uninhibited: normal specimens are 42 to 66 per cent depressed by 10^{-3} M HCN, depending upon their age.

It is perhaps more than coincidental that two of the three tissues found

in the present study to be completely cyanide-insensitive have been reported to contain a high concentration of vanadium. Vanadium does not combine with cyanide (15) and a catalytic system dependent upon this metal for electron transfer should be unaffected by HCN. Vanadium is almost absent from sea water and it was possible to determine only a trace of it in the residue from 200 liters (16). However, tunicates have been known for a long time to contain this metal (17–19), and the same species of sea cucumber used in the present experiments was found to have a vanadium content of 0.12 per cent of the dry weight when collected at the Tortugas (20). Spectrographic analysis of a number of other kinds of biological materials has shown that vanadium is usually absent or if it is present it is found in extremely low concentration (21). This scarcity of the metal suggests that the correlation of high vanadium content and cyanide insensitivity in the tunicates and sea cucumbers is possibly more than coincidental. (Although the increased oxidation of phospholipids by liver suspension containing added sodium metavanadate was inhibited by cyanide (22), this may have been due to involvement of other heavy metal systems.) The possibility of vanadium participating in the cellular respiration of these cyanide-insensitive forms as an electron-transfer catalyst will be more thoroughly investigated.

The great range and the thousandfold difference in the extremes of the K values listed in Table II may be indicative of some as yet unknown variable in the cellular respiration system. An adequate explanation of this difference in the sensitivity of various animals to HCN is lacking at present.

When inhibition data are plotted logarithmically as in Fig. 5 a regular variation of respiration with the concentration of the inhibitor results in a straight line. If the slope of the line is 1 it is an indication that one mole of the inhibitor is combined with one mole of the enzyme (4). Since most of the curves do give slopes approximating 1 it is evident that a 1-to-1 relationship of enzyme and inhibitor is the usual condition. It may be significant that in all the anomalous cases a high proportion of the respiration is unaffected by cyanide, indicating probably the participation of another type of system. Deviations from a slope of 1 may result if there is inhibition of more than one metabolic step, or if the kinetics of respiration do not depend only upon the concentration of the enzyme (23). Fisher (24) lists slope values from 0.52 to 1.6 for data obtained from various types of material by several investigators; difficulties in controlling cyanide concentrations during measurement periods may have contributed to this wide variation.

As shown in Fig. 6 and Table II the respiration of the unfertilized egg of the sea urchin, *Tripneustes*, is almost completely inhibited by 10^{-4}M HCN. This is comparable to the situation found in *Arbacia*, where, although it had been supposed that the unfertilized egg was insensitive to cyanide, improved techniques have shown that the respiration is at least 60 per cent depressed by 10^{-4}M HCN (25).

SUMMARY

A study of the metabolism of Bermuda marine invertebrates at 25°C. shows that the respiratory rates of many of the tissues approximate those of vertebrate tissues at the same temperature. There is no apparent correlation between respiratory rate and phylogenetic development: tissues from some of the simpler forms use as much oxygen per unit weight as those from certain of the more highly developed animals.

Cyanide inhibition experiments reveal a great variation in the amount of oxygen consumption which is dependent upon sensitive heavy metal systems. Three types of tissues, the jellyfish *Cassiopea frondosa*, the branchial tree of the sea cucumber, *Stichopus möbii*, and two kinds of tunicates, were completely unaffected by even 10^{-2}M HCN. Other tissues such as sea urchin sperm, squid gills, and lobster nerve and muscle were almost completely inhibited by much lower concentrations. Most of the materials retained 20 to 40 per cent of the normal respiratory rate in 10^{-2}M HCN. The possibility that vanadium may play a part in the oxidation-reduction systems of the completely resistant animals is discussed.

There is a thousandfold variation in the concentration of cyanide required to produce 50 per cent inhibition of respiration in the different tissues. Sea urchin sperm is 50 per cent inhibited by 10^{-6}M HCN: the sea fan requires 10^{-3}M for the same effect. Other tissues lie at intermediate points.

When the logarithm of the ratio of the inhibited to the uninhibited respiration is plotted against the concentration of cyanide the resulting line has a slope which in most cases approximates 1. This indicates that one mole of enzyme ordinarily combines with one mole of inhibitor.

Eggs of the sea urchin, *Tripneustes esculentus*, show a three- to fivefold increase in the rate of oxygen uptake on fertilization. The respiration of both the fertilized and unfertilized eggs is almost entirely inhibited by 10^{-4}M HCN. Cell division in the fertilized eggs is blocked by somewhat less than 10^{-5}M cyanide, a concentration which reduces respiration to 40 per cent of the normal level.

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