compared with standard commercial enamels. The results are shown in Fig. 5.

Reflectances between 60 and 70% were obtained with three of the experimental compositions applied to thicknesses of 0.012″–0.014″. Commercial enamels opacified with zirconia or titania, however, were more opaque at lower weights of application.

References

1. BLAU, H. H. *Ind. Eng. Chem.*, **25**, 848 (1933).
2. KREIDL, N. J., and WEYL, W. A. *Glass Ind.*, **21**, (2), 57 (1940).
3. ANDREWS, A. I., and BREAN, J. G. *J. Am. Ceram. Soc.*, **16**, 325 (1933).
4. ANDREWS, A. I., CLARK, G. L., and ALEXANDER, H. W. *Ibid.*, **14**, 634 (1931).
5. HARSHAW, W. J., and STILLWELL, A. G. Opacifier for Vitreous Enamels. U. S. Patent 2,200,170 (May 7, 1940).
6. KING, B. W., JR. *Finish*, **2**, (9), 18 (1945).
7. ANDREWS, A. I. *Enamels.* Champaign, Ill.: Twin City Pub., 50 (1935).
8. KINZIE, C. J., and COMMONS, C. H., JR. *J. Am. Ceram. Soc.*, **17**, 283 (1934).
9. BINNS, C. F. *Trans. Am. Ceram. Soc.*, **5**, 50 (1903).
10. ANDREWS, A. I. *Op. cit.*, 181, 191, 195.
11. HARSHAW, W. J., and STILLWELL, W. A. Vitreous Enamel Opacifier. U. S. Patent 2,033,707 (Mar. 10, 1936).
12. HERBERT, C. J., and BATEMAN, L. A. Manufacture of Opaquing Agents. U. S. Patent 2,306,354 (Sept. 16, 1940).
13. LANG, H. *Glashütte*, **64**, (8), 121 (1934).
14. HALDY, N. L., WRIGHT, J. H., and TODD, F. C. *J. Am. Ceram. Soc.*, **30**, 153 (1947).

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Some Patterns of the Respiratory Pigments of Ascites Tumors of Mice

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Inasmuch as we are now able to record accurately the visible and ultraviolet spectra and reactions of the pyridine nucleotides and the cytochromes of various tissue homogenates and intact cells (*1*), it is of interest to compare the pattern of the respiratory pigments of such normal cells with those of neoplastic cells. The ascites tumor, as described recently by Klein (*2*), is the most suitable type of material for our studies since viable and transmissible tumor cell suspensions are obtained in adequate quantities from a single mouse, and the intact cells are studied directly by our methods, without the need for homogenization, as would be necessary with solid tumors. A further advantage of these cells is that they are maximally dedifferentiated.[2]

The mice[3] were sacrificed on the sixth to fifteenth day after inoculation, the tumor cells were freed from erythrocytes by osmotic shock (differential lysis), and the hemoglobin was largely removed by subsequent washing at 0°. The tumor cell counts then averaged about 5×10^4/mm³ for the Ehrlich and Krebs 2 suspensions, and the Q_{O_2} was about 3 at 25° with glucose as a substrate. Successful results were obtained with three tumor cell types: Ehrlich, Krebs 2, and *dba* thymoma ascites (*3*).

The changes in optical density measured here are those that occur upon the exhaustion of the oxygen dissolved in the cell suspension. The normal respiration of our cell suspensions causes anaerobiosis to occur in several minutes. At that time those pigments oxidized by respiratory activity become reduced. A typical experiment is represented by Fig. 1, where the

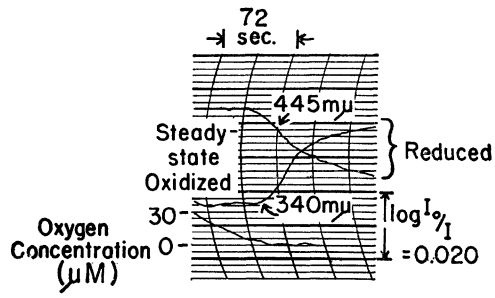

FIG. 1. An illustration of the spectrophotometric measurement of optical density changes in a respiring ascites tumor cell suspension that are coincident with the termination of the oxidase activity. An upward deflection of the traces at 340 mμ represents an increase of optical density, whereas a downward deflection at 445 mμ represents an increase of optical density; *dba* thymoma, cell count, 1.7×10^5/mm³. (Expt 921–12.)

respiration is automatically recorded from polarographic analyses with a platinum electrode. There is no deflection of the spectrophotometric trace in the steady-state oxidized system, but, upon exhaustion of the oxygen, there is a reduction of cytochrome a_3 (a downward deflection of the trace at 445 mμ corresponds to an increase of optical density) and of pyridine nucleotide (an upward deflection of the trace at 340 mμ corresponds to an increase of optical density). The complete spectrum representing the difference between the reduced and the steady-state oxidized condition of the respiratory pigments is obtained by repeating the experiment of Fig. 1 at various wavelengths or by using a continuous recorder (*4, 5*).

Our results for Krebs 2 ascites tumor cells are given in Fig. 2. These spectra are dominated by cytochrome *c*; the strong visible bands at 549.5, 520, and the Soret

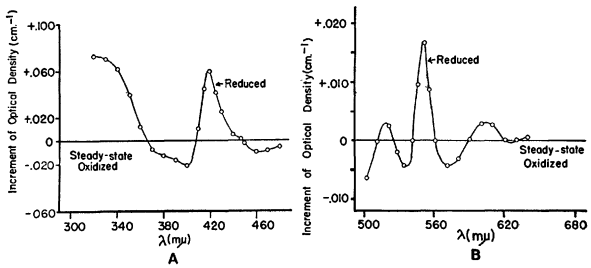

FIG. 2. The spectra representing the difference between the reduced and steady-state oxidized respiratory pigments of Krebs 2 ascites tumor; cell count, 5.8×10^4/mm³. (Expt 921–4.)

[1] Lalor Foundation predoctoral fellow.

[2] Dale Coman kindly suggested the use of ascites tumor.

[3] T. S. Hauschka, of the Cancer Research Institute, Fox Chase, Pa., very kindly supplied us with mice inoculated with Ehrlich, Krebs 2, and *dba* thymoma ascites.

TABLE 1

COMPARISON OF THE PATTERN OF RESPIRATORY PIGMENTS OF TUMOR AND OTHER CELLS

Material	Expt No.	$K_4 = \frac{\mu MO_2/sec}{\Delta D_{445-480}}$ (25°)	Relative optical density changes*				DPNH
			a	*b*	*c*	a_3	
Keilin and Hartree heart muscle preparation (*6*)	880b	49	1	0.6	0.8	9	—
Rabbit heart sarcosomes†	923c	14	1	0.8	0.9	11	—
Fly muscle sarcosomes‡	910e	12	1	0.6	1.5	9	—
Bakers' yeast cells	876a 918f	160	1	1.7	2.7	11	60
Ehrlich ascites§‖	921-8	37	1	< 0.5	4	12	—
Krebs 2 ascites§‖	921-4	40	1	< 0.5	4	6	13
dba thymoma§	921-12	32	1	0.4	2	6	11

* In this table we have evaluated the optical density changes from the peak to the trough of the absorption band—cytochrome *a*: $\Delta D_{605}-\Delta D_{650}$; cytochrome *b*: $\Delta D_{561}-\Delta D_{570}$; cytochrome *c*: $\Delta D_{550}-\Delta D_{541}$; cytochrome a_3: $\Delta D_{445}-\Delta D_{480}$.

† Prepared according to unpublished data of E. C. Slater.

‡ Prepared from blowflies kindly furnished by M. I. Watanabe, Quartermaster Corps, Philadelphia.

§ The tumor cells were suspended in a medium consisting of 0.85% sodium chloride, 0.025% potassium chloride, 0.030% calcium chloride, to which 0.1% glucose was added.

‖ The value of $\Delta D_{445}-\Delta D_{480}$ was corrected for cytochrome-*c* absorption.

band at 419 mμ identify this pigment with certainty and indicate it to be present in a relatively large concentration. Cytochrome *a* is observed at 605 mμ, and cytochrome a_3 as the small shoulder on the Soret band at 445 mμ. Reduced pyridine nucleotide absorbs below 340 mμ.

Since these ascites cells do not retain the characteristics of the tissue of origin (*2*), we have chosen to compare their respiratory pigments with those of muscle and yeast cells, of high respiratory activity, as given elsewhere (*1*). Such a comparison is aided by the data of Table 1, in which the relative intensities of the absorption bands of the 4 cytochromes and pyridine nucleotides of 4 types of normal cells and 3 types of ascites tumors are compared. In addition the quantity, $K_4 = \mu MO_2/sec/\Delta D_{445-480}$, is included. This quantity is our criterion of the intensity at which cytochrome oxidase is operating and is related to the actual turnover number of the enzyme. (Since the respiration rate in these tumor cells is considerable, the respiratory pigments may already be partly reduced in the steady-state oxidized condition [*1*]. But, in view of the values of K_4 and our studies of heart muscle preparations, only about 10% of cytochromes *a* and a_3, and 30% of cytochrome *c*, would be so reduced.)

Turning our attention first to the consistencies of the patterns, we find that the quotient of the absorption change at 445 mμ, due mainly to cytochrome a_3, and of the absorption change at 605 mμ, due mainly to cytochrome *a*, varies from 6 to 12 and is fairly close to the average value of 10 for normal cells. Studies of the effect of carbon monoxide upon the absorption bands at 445 and 605 mμ in the tumor cells verify the approximately normal relative values of cytochromes a_3 and *a*.

A second consistency is seen in the values of K_4 which indicate that the turnover number of cytochrome a_3 is a rather usual value; the amount of cytochrome oxidase present is working at a moderate rate, slow compared to yeast, but fast compared to mitochondrial preparations from heart muscle.

Reduced pyridine nucleotide is readily detected in these cells in a reasonable amount.

There are, however, some significant inconsistencies in these patterns. Cytochrome *b* is a pigment whose absorption band is conspicuously absent from the spectra of Fig. 2; no shoulder appears on the 550 mμ absorption band of cytochrome *c*, nor is there any peak at 430 mμ. A more sensitive test for cytochrome *b* is to add antimycin *a* (*7*) to the aerated suspension and thereby to uncouple cytochrome *b* from the other cytochromes and permit its aerobic reduction. Such a test reveals a barely detectable absorption band in Ehrlich and Krebs 2 cells and points to their large relative deficiency of cytochrome *b*. In *dba* thymoma, we were able positively to identify cytochrome *b*.

Cytochrome *c* shows the most marked inconsistency, and the amount of this pigment is abnormally great in all samples tested. The relative content of cytochrome *c* found in the Ehrlich and Krebs 2 tumor cells actually exceeds that of the very highly respiring yeast cell.

In summary, we can state that the respiratory activity of ascites tumor cells, mediated by cytochromes a_3 and *a*, closely follows the pattern of normal muscle and yeast cells. Cytochrome *c* is present in an unusually large amount relative to cytochrome *a* and a_3, and cytochrome *b* is difficult to detect at all—the cytochrome *c* to cytochrome *b* ratio in these ascites tumors is over fourfold greater than that of mammalian heart muscle.

In comparing our results on the relative content of cytochrome *c* and cytochrome a_3 of these tumors with those obtained by previous workers on solid tumors, it is of interest to note that they concluded that the disparity of these two components is of just the opposite sense, cytochrome *c* being relatively defi-

cient with respect to cytochrome oxidase (*8*). We believe that our direct spectroscopic study of the pigments of the intact, freely suspended, respiring tumor cells is somewhat more decisive than the various indirect assay methods that have led to the earlier conclusions.

References

1. CHANCE, B. *Nature*, **169**, 215 (1952).
2. KLEIN, G. *Cancer*, **3**, 1052 (1950).
3. HAUSCHKA, T. S. (To be published.)
4. CHANCE, B. *Rev. Sci. Instruments*, **22**, 619 (1951).
5. YANG, C. C. Paper presented at mtg. Inst. Radio Engrs. (Mar. 2, 1952).
6. KEILIN, D., and HARTREE, E. F. *Biochem. J.*, **41**, 500 (1947).
7. AHMAD, K., SCHNEIDER, H. G., and STRONG, F. M. *Arch. Biochem.*, **28**, 281 (1950).
8. GREENSTEIN, J. P. *Biochemistry of Cancer*. New York: Academic Press, 269 (1947).

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The State of Catalase in the Respiring Bacterial Cell[1]

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The physiological role of catalase has remained obscure because the two substances necessary for catalase action (a substrate such as peroxide and a hydrogen donor such as an alcohol, or nitrous or formic acid [*1*]) have not been proved to be present simultaneously in adequate concentrations in the respiring cell (*1*). In this paper we show the existence of both types of substances and, furthermore, show how the steady-state peroxide concentration can be regulated by the addition of suitable hydrogen donors, a point of some importance in the control of peroxide produced within the cell. The paper also provides the first demonstration of an enzyme-substrate compound in action *in vivo*.

By means of sensitive spectrophotometric methods (*2*) we can now study the spectra and kinetics of catalase and cytochrome in respiring suspensions of *Micrococcus lysodeikticus* (*3*).[2] We find catalase in the form of its primary hydrogen peroxide complex (complex I) (*4*) in the aerobic bacterial cells. The complex is identified by its specificity for donors such as nitrous and formic acid and by its absorption spectrum, as previously determined by *in vitro* studies of the pure crystalline enzyme (*5*).

When catalase is continuously supplied with hydrogen peroxide from an oxidase system, we have shown *in vitro* that the steady-state concentration of complex I may be considerably reduced by the addition of a donor (*6*). The same experiment is here repeated with intact respiring cells, as shown in Fig. 1, *A*. The recording spectrophotometer is responsive

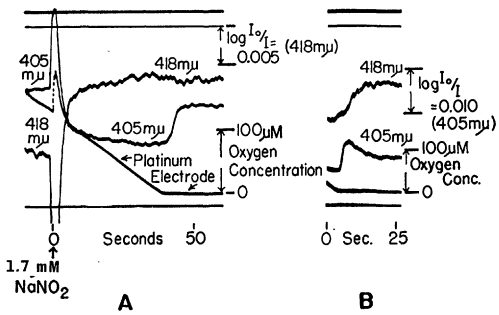

FIG. 1. *A*: Spectrophotometric recording of the addition of nitrite to a respiring suspension of *M. lysodeikticus*. The respiration of the bacteria is indicated by the linear decrease of the polarographic trace. The double beam spectrophotometer was set at 405 mμ and 418 mμ and records an increase of optical density as an upward deflection of the 418 mμ trace and a downward deflection of the 405 mμ trace (to avoid trace superposition). Cells suspended in a phosphate medium of pH = 7.0 with 2.7 mM glucose present; 1.7 mM nitrite added. (Expt 922b.) *B*: A similar record, but nitrite is omitted. (Expt 922a.)

to a change in the concentration of complex I at both 405 mμ (the peak of the complex I band) and 418 mμ (a wavelength at which the cytochromes of the bacteria do not interfere with the measurement of complex I). Both traces show an increase of optical density upon addition of 1.7 mM nitrite (or in other tests, a larger formate concentration), as would be expected when the steady-state concentration of complex I is reduced. (The absorption band of free catalase is higher than that of complex I [*5*, Fig. 3].) Upon exhaustion of the oxygen, the cytochromes are reduced, as shown by the decrease of optical density at 405 mμ. In the absence of added donor, complex I is present aerobically, and rapidly decomposes upon exhaustion of the oxygen, as clearly shown by the trace at 418 mμ in Fig. 1, *B*. These two experiments suggest that complex I is present in respiring cells and that adequate endogenous donor is present to cause its rapid decomposition when the cells become anaerobic.

The spectrum of the change in absorption caused by the addition of a catalase donor to aerobic cells is obtained by repeating the experiment of Fig. 1, *A* at various wavelengths or by using a sensitive recorder (*7*) as shown by Fig. 2, Curve *A*. This difference spectrum is identical to our *in vitro* result for complex I (*5*). For comparison, the cytochrome absorption is recorded in Curve *B*. Further proof of our conclusion is afforded by adding methyl hydrogen peroxide to the aerated cells, which greatly decreases the absorption (Fig. 2, *C*) as a result of further binding of catalase hematin as complex I of methyl hydrogen peroxide, in agreement with our *in vitro* studies (*5*, Fig. 4). A comparison of Curves *A* and *C* shows that about 1.6 molecules of hydrogen peroxide are bound to the 4 hematins of bacterial catalase *in vivo*, in accord with the *in vitro* calcula-

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[2] The fact that this bacterium has an unusually large catalase content means that a higher level of free peroxide concentration is required to reach the saturation value of complex I.