phase relations, strongly suggest that the $(\bar{1}01)$ lamellae are due to exsolution, other modes of origin of these lamellae are possible.

Published chemical analyses and recent electron-probe analyses indicate that the anthophyllite and cummingtonite crystal structures contain very little calcium—probably less than 2 percent CaO by weight. We expect, however, that the $P2_1/m$ clinoamphiboles, which are analogous to the pigeonite clinopyroxenes, are richer in calcium.

MALCOLM ROSS
J. J. PAPIKE
U.S. Geological Survey, Washington, D.C. 20242
PAUL W. WEIBLEN
Department of Geology and Geophysics, University of Minnesota, Minneapolis

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9. The error in total counts is expected to be less than 3 percent two-thirds of the time; within 6 percent 95 percent of the time.
10. These rocks are being studied by H. L. James and K. W. Shaw, U.S. Geological Survey; we thank them for letting us examine these specimens and for advice on the petrogenesis. Also we thank others (*11–14*) for generously furnishing the necessary amphibole specimens.
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Carcinogens 3,4-Benzpyrene and 3-Methylcholanthrene: Induction of Mitochondrial Oxidative Enzymes

Abstract. *Sections of liver from rats injected with 3,4-benzpyrene and 3-methylcholanthrene, when incubated in mediums specific for the histochemical demonstration of mitochondrial oxidative enzymes, show greater activity of several of these enzymes than do sections from control rats. This observation was confirmed by comparison of the staining of mitochondria isolated from the control and from "induced" rats. The fact that an inhibitor of protein synthesis, actinomycin D, effectively diminished the stimulation provided evidence that the stimulation of activity is due to an increase in enzyme synthesis, generally called induction.*

Adaptive changes whereby pharmacologically active substances stimulate metabolic activity, by enhancing the physiological synthesis of additional amounts of enzymes, may alter the duration and intensity of drug action in animals and man (*1*). Acceleration of the metabolism of various lipid-soluble drugs by liver microsomal enzymes responsible for inactivation reactions has been demonstrated (*1*). Thyroid hormone has been shown biochemically (*2*) to stimulate the activity of several rat-liver microsomal enzymes, as well as to enhance incorporation of amino acid into protein by liver microsomes. Phenobarbital, 3,4-benzpyrene, and 3-methylcholanthrene also have been shown (*3*) to enhance markedly this latter function of the microsomal liver fraction. Adaptive stimulation of mitochondrial oxidative enzymes by thyroxine has also been demonstrated (*4*) biochemically in investigations of the mechanism of control of basal metabolic rate by thyroid hormone. Further similarity between the different types of inducing agents is indicated by a report (*5*) of the probable enhancement of mitochondrial oxidative enzymes in livers of rats treated with phenobarbital. Such similarity is not unexpected, since concomitantly with enhancement of either the microsomal or the mitochondrial oxidative enzymes there occurs proliferation of the protein of these organelles; although the microsome is primarily responsible for protein synthesis within the cell, the mitochondrion has its own capability (*6*) for production of protein enzymes.

We tried to demonstrate the enhancement, in tissue sections, of mitochondrial oxidative enzymes with chromogenic histochemical methods specific for these enzymes. Use of an appropriate substrate and the ditetrazolium salt nitro blue tetrazolium (nitro-BT) (*7*) permits deposition of diformazan pigment at the intramitochondrial sites of dehydrogenase activity (*8*). Another reagent, 3,3′-diaminobenzidine, which undergoes oxidative polymerization, accurately delineates the localization of cytochrome *c* of the cytochrome oxidase triplet to mitochondria in heart, liver, and kidney (*9*).

We confirmed histochemically an increase in mitochondrial α-glycerophosphate dehydrogenase, malic dehydrogenase, succinic dehydrogenase, dihydronicotinamide adenine dinucleotide (DPNH) diaphorase, and cytochrome oxidase in tissue sections of livers of rats made hyperthyroid. We also confirmed histochemically the earlier observation (*5*), on isolated mitochondria, of the enhancement of mitochondrial malate dehydrogenase and succinic dehydrogenase in livers of rats injected with phenobarbital; the livers of these rats also showed an increase in cytochrome oxidase activity.

The differences in absorbance of the histochemical pigments observed in control and in experimental tissue sections were reproducible. Moreover, the histochemical methods used permitted direct observation of the particular organelles involved in the reaction. Classical biochemical methods such as measurement of oxygen utilization (*10*) require prior isolation of the organelles under study by differential centrifugation (*11*) of tissue homogenate. We then applied our methods to study of liver sections of rats given the doses of 3,4-benzpyrene and 3-methylcholanthrene that had been used to "induce" microsomal enzymes (*1*). A definite increase was observed although the profile of increased mitochondrial dehydrogenase activity differed from that obtained by stimulation with thyroid hormone or phenobarbital.

Table 1 summarizes the histochemical observations on rat livers for several dehydrogenases and cytochrome oxidase after stimulation by triiodothyronine (T_3), phenobarbital, 3,4-benzpyrene, and 3-methylcholanthrene; we have graded the intensities of staining,

Table 1. Comparison of color intensities in 6-μ fresh-frozen sections of rat liver. The dehydrogenases (dehy.) and DPNH diaphorase were determined (*8*) with the appropriate substrate and nitro-BT. Cytochrome oxidase was determined by the 3,3′-diaminobenzidine method (*9*). For each enzyme, reagent concentrations and incubation times were adjusted so that the control adult male (Wistar) rat liver gave a reading of 1. All inducing agents were administered intraperitoneally. Daily dosage per 100 g of rat: triiodothyronine sodium salt (T_3), 100 μg; phenobarbital sodium, 15 mg; 3,4-benzpyrene, 0.7 mg; 3-methylcholanthrene, 8 mg. The T_3 and phenobarbital were injected in normal saline; the control rats received saline intraperitoneally. The benzpyrene and methylcholanthrene were injected in 0.1 ml of sesame oil; the controls were given 0.1 ml of sesame oil intraperitoneally. Approximately 19 hours after the last injection, the rats were stunned and decapitated. Each value is based on results from at least four rats. In assigning integral values for color intensity we attempted to adhere to the following values for corresponding increases in intensity: +1, < 50 percent; +2, 50 to 100 percent; +3, 100 to 150 percent; +4, 150 to 200 percent.

Enzyme	Color intensity after injection of:						
	T_3			Phenobarbital		Benzpyrene (2-day)	Methylcholanthrene (2-day)
	2-Day	3-Day	4-Day	2-Day	3-Day		
α-Glycerophosphate dehy.	+3	+3	+4	+2	+2	+2	+1
Malic dehy.	+2	+2	+3	+2	+2	+1	+1
Succinic dehy.		+3	+3	+2		+2	+1
Lactic dehy.		+3	+3	+3		+3	+2
Glutamic dehy.		+3	+3	+3		+2	+2
β-Hydroxybutyric dehy.		−1				+4	+3
DPNH diaphorase						+4	+4
Cytochrome oxidase	+2	+3	+3	+2	+2	+3	+3

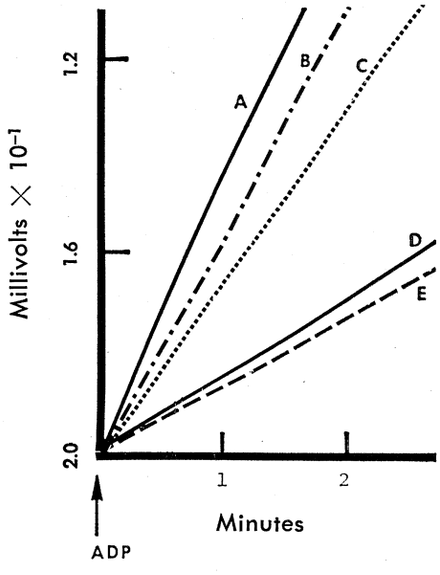

Fig. 1. Composite of initial portions of oxygen-uptake curves for mitochondria isolated from equal weights of liver of control rats and injected rats. The relation between voltage and oxygen content is linear in the system. To the reaction vessel at 30°C was added 0.2 ml of mitochondrial suspension (mitochondria from 1 g of liver, reconstituted to 1 ml with 0.25*M* sucrose) and 1.8 ml of 0.25*M* sucrose containing 5 m*M* $MgCl_2$, 25 m*M* K_2HPO_4, and 1.0 m*M* tris-HCl buffer, *p*H 7.4. After temperature equilibration (approximately 1 minute), 20 μl of 2*M* succinate or 2*M* β-hydroxybutyrate was added, followed by 20 μl of 0.1*M* adenosine diphosphate. The animals were treated as for Table 1. Curve *A*: succinic dehydrogenase activities of controls and of animals injected with benzpyrene or methylcholanthrene. Curves *B–E*: β-hydroxybutyrate dehydrogenase activity in benzpyrene-injected rat (*B*), methylcholanthrene-injected rat (*C*), rat injected with methylcholanthrene and actinomycin D (*D*), and control rat (*E*).

observed visually in tissue sections of equal thickness, and assigned integral values from 0 to +4. The increase in activity observed in liver sections after 3,4-benzpyrene was also noted with isolated mitochondria (*11*) stained histochemically with essentially the same procedures; intensities of staining in pellets of equal volume were compared after centrifugation in a microcentrifuge (*12*). The differences between normal and injected rats were best seen when the incubation time and concentration of reagent were kept lower than those used for tissue sections.

Further confirmation of the validity of the observed induction was obtained by comparison of the rates of oxygen consumption (*13*) (Fig. 1) by mitochondria isolated from the livers of control rats and of rats injected with benzpyrene or methylcholanthrene; β-hydroxybutyrate or succinate was used as substrate in the presence of phosphate and phosphate acceptor (adenosine diphosphate).

The differences in profile of enzyme stimulation by various agents suggests that the changes we observed in mitochondrial oxidative enzymes are not due to a generalized toxicity phenomenon. In preliminary experiments, actinomycin D (an inhibitor of DNA-dependent synthesis of messenger-RNA) (*14*) at 40 μg/kg diminished the increase in activity caused by simultaneous administration of benzpyrene or methylcholanthrene. The increases in activities of DPNH diaphorase and lactic dehydrogenase were almost completely blocked by actinomycin D; cytochrome oxidase stimulation was partially blocked; and β-hydroxybutyric dehydrogenase stimulation was diminished only slightly in the case of benzpyrene, but almost completely blocked in the case of methylcholanthrene (Fig. 1).

Ultrastructural histochemical studies, with the osmiophilic reagents (*15*) 3,3′-diaminobenzidine (*9*) for cytochrome oxidase and thiocarbamylnitro blue tetrazolium (*16*) for the dehydrogenases, may yield further information on the relation of induced enzyme profile to ultrastructure.

NICHOLAS ZENKER
Department of Pharmaceutical Chemistry, University of Maryland School of Pharmacy, Baltimore
JACOB S. HANKER
YOSHIHISA MORIZONO
CHANDICHARAN DEB
ARNOLD M. SELIGMAN
Departments of Surgery, Sinai Hospital of Baltimore, and *Johns Hopkins University School of Medicine, Baltimore, Maryland*

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