After 2 weeks the rats were killed and collagen was analyzed (see Table 2).

All animals receiving penicillamine (groups 2 to 4) developed decrease in tensile strength of skin and increase in the soluble collagen fraction. Oral supplementation of copper in low concentration (group 2), and in a concentration sufficiently high to be toxic and retard growth (group 3), failed to prevent development of the drug-induced collagen defect.

In the first series of experiments, the penicillamine-induced abnormalities in soft-tissue collagen occurred with only a moderate decrease in total body copper, while a far greater degree of copper depletion, produced by the copper-deficient diet, failed to result in similar changes. This finding suggested that the mechanism of action of penicillamine on collagen was not due to copper-depletion by chelation.

In the second experiment the rats received orally large supplements of copper, the penicillamine being administered parenterally to avoid formation of a penicillamine-copper chelate in the gastrointestinal tract, which would have rendered the drug unavailable. The results show that, even with massive doses of copper, administration of penicillamine produced the expected alterations in dermal collagen. The penicillamine-induced collagen defect is believed to be independent of copper, probably being related to the particular sulfhydryl reactivity of the molecule—perhaps by formation of a stable thiazolidine compound with aldehyde groups that would interfere with the cross-linkage of the collagen fibrils (7).

ISRAELI A. JAFFE, PARVIN MERRIMAN
DAVID JACOBUS
Department of Medicine, New York Medical College, New York, and
Walter Reed Army Institute for Research, Washington, D.C. 20012

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Hypertrophic, Hypoactive Smooth Endoplasmic Reticulum: A Sensitive Indicator of Hepatotoxicity Exemplified by Dieldrin

Abstract. *Rats with hypertrophic smooth endoplasmic reticulum (ER) and increased activities of the drug-handling enzymes induced by dieldrin were stressed with larger doses of the pesticide. The activity of the drug-handling enzymes was thus reduced, but liver weight, smooth ER, and P-450 hemoprotein remained elevated. While no changes were apparent by light microscopy, the hypertrophic, hypoactive smooth ER was recognized as tight clusters of tubular membranes associated with abnormalities of the mitochondrial membrane. Similar but not identical morphologic changes were noted in human liver diseases associated with hepatic insufficiency. Hypertrophic, hypoactive smooth ER may indicate transition from adaptation of injury, and can be used as a sensitive parameter of toxicity.*

Ingestion of any of a wide variety of substances [including drugs (*1*), alcohol (*2*), and food contaminants such as pesticides (*3*)] effects enlargement of the liver and hypertrophy of hepatocellular smooth endoplasmic reticulum (ER). The ER is the site of drug-handling enzymes such as aniline hydroxylase and *p*-nitroreductase. These enzymes are part of a nicotinamide-adenine dinucleotide phosphate–dependent electron-transfer chain, the terminal component of which is the P-450 hemoprotein (*4*).

Usually the hypertrophic smooth ER is associated with increased activity of the drug-handling enzymes (*5*), and may thus reflect adaptation. However, it is also found in the presence of recognizable hepatic injury, such as experimental biliary obstruction (*6*), after administration of a carcinogen (*7*), and in diseases of the human liver in which detoxification is known to be impaired. Thus the following questions are raised: (i) Can hypertrophic smooth ER be hypofunctioning? (ii) Does hypertrophic, hypofunctioning smooth ER reflect toxicity, and does it precede changes detectable by light microscopy? (iii) Are there associated alterations of other organelles?

Rats in which tolerance to dieldrin had been induced were stressed with larger doses of this pesticide, and ultrastructural changes were compared with the activity of the drug-handling enzymes and the level of P-450 hemopro-

Table 1. Effects of administration of dieldrin on liver weight, on contents of microsomal protein and P-450 hemoprotein, on the activities of microsomal drug-handling enzymes, on mitochondrial proline oxidation, and on oxidative phosphorylation. Rats received dieldrin at 0.2 mg/100 g daily for 28 days, and at 0.5 mg/100 g daily during the following 24 days. Phases: NSS, new steady state; Decom., decompensation.

Item	Days on dieldrin			
	0 (Control)	14 (NSS)	28 (NSS)	52 (Decom.)
Liver weight (g/100 g)	3.35 ± 0.18§	4.03 ± 0.23	3.83 ± 0.025	4.00 ± 0.30
Microsomal protein (mg)*	71.0 ± .8‖	99.0 ± 12.0	96.0 ± 14.0	102.0 ± 11.7
Aniline hydroxylase (mμmoles)†	10.8 ± 1.3§	20.2 ± 4.2	17.5 ± 4.1‖	12.4 ± 1.8
p-Nitroreductase (mμmoles)†	105.0 ± 17.0§	196.0 ± 37.0	186.0 ± 17.0§	99.0 ± 9.0
P-450 hemoprotein (mμmoles)‡	0.55	1.01		1.10
Aniline hydroxylase: P-450	19.6	20.0		11.3
Proline oxidase (μmoles)*	2.53 ± 0.21	2.46 ± 0.16	2.49 ± 0.24§	1.76 ± 0.33
Oxidative phosphorylation	2.25	2.11	1.99	1.18

* Mean ± standard deviation (S.D.) relative to total liver per 100 g of body weight (ten determinations). † Enzyme activity per milligram of microsomal protein, ± S.D. (ten determinations). ‡ Average of four determinations. § Difference between the mean and the following mean is statistically significant ($p > .001$). ‖ Difference between the mean and the following mean is statistically significant ($p > .01$).

tein. Female Sprague-Dawley rats having intial body weights of 150 g were given dieldrin intraperitoneally in daily doses of 0.2 mg per 100 g of body weight in 0.01 ml of mineral oil for 28 days before the dose was increased to 0.5 mg/100 g; the control group received mineral oil only. Portions of each liver were homogenized in 0.25*M* sucrose at 0°C. The microsomal fraction was isolated by ultracentrifugation, and the activities of aniline hydroxylase and *p*-nitroreductase, and the concentration of P-450 hemoprotein, were determined in a manner reported (*2*). Protein was determined by the method of Lowry *et al.* (*8*), oxidative phosphorylation was measured polarographically by the method of Brierley (*9*), and activity of proline oxidase was measured by a reported method (*10*).

Liver tissue obtained at the time of death was fixed in buffered osmium tetroxide, dehydrated, and embedded in epoxy resin; sections were cut and stained with periodic acid–Schiff and toluidine blue for light microscopy. After lobular localization, thinner sections were cut and stained with lead citrate for electron microscopy. Biopsy material from patients with various liver diseases was surveyed for appearance of the ER and mitochondria.

As had been shown (*3*), the administration of dieldrin produced enlargement of the liver, hypertrophy of the smooth ER (Fig. 1), increase in microsomal protein, and parallel increase in the activities of aniline hydroxylase, *p*-nitroreductase, and P-450 hemoprotein. The enzyme activities per mole of available P-450 hemoprotein remained unchanged. The highest level of activity of the processing enzymes was reached after 14 days (phase of induction); this point was followed by a "new steady state" during which the elevations of the enzymes and of P-450 hemoprotein were maintained. The new steady state indicates increased tolerance. While five consecutive doses of dieldrin at 0.5 mg/100 g, given to previously untreated rats, caused 70 percent mortality, animals survived 25 consecutive doses at 0.5 mg/100 g if this dosage began after the new steady state had been established by dosage at 0.2 mg/100 g for 28 days.

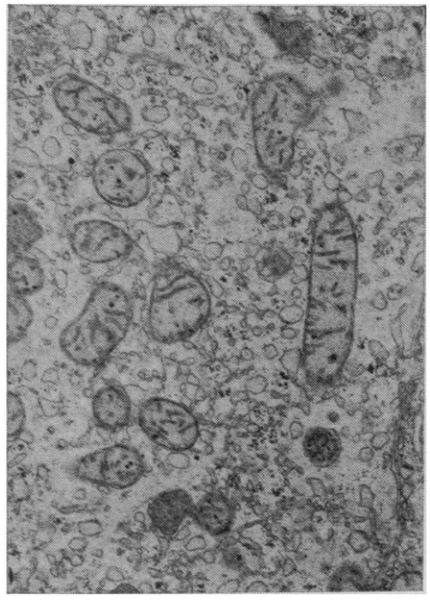

Fig. 1 (left). Portion of hepatocyte of rat given dieldrin for 9 days, showing tubular and vesicular smooth endoplasmic reticulum with little interspersed glycogen (× 7500).

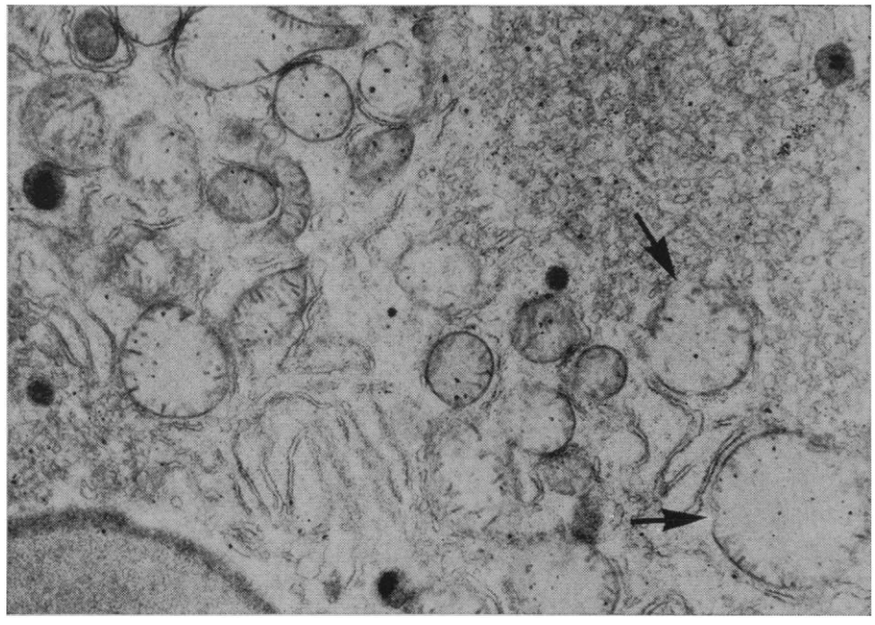

Fig. 2 (below). Cluster of tightly packed tubules of hepatic smooth endoplasmic reticulum, and focal loss of outer mitochondrial membranes (arrows). Rats were given dieldrin first at 0.2 mg/100 g for 28 days and then at 0.5 mg/100 g for 24 more days (× 12,500).

The duration of the new steady state varies with different substances; that induced by phenobarbital (*11*), or by the smaller dose of dieldrin, can be maintained almost indefinitely, and the alterations are fully reversible on withdrawal. The larger doses of dieldrin failed to produce changes detectable by light microscopy, but the activities of the drug-handling enzymes declined progressively.

During this phase of decompensation the smooth ER remained hypertrophic, and the microsomal protein and P-450 hemoprotein concentrations remained elevated; the enzyme activities per mole of available P-450 hemoprotein decreased. Mitochondrial proline oxidase activity and coupled oxidative phosphorylation, which had been maintained at control levels throughout the steady state, also declined (Table 1). In the hepatocytes much of the excess smooth ER formed tightly packed clusters of tubular membranes with no glycogen and little hyaloplasm, and some mitochondrial membranes were focally separated or missing, or were interdigitated with those of adjacent mitochondria (Fig. 2). The phase of decompensation, therefore, was represented by hypertrophic but hypoactive smooth ER which may serve as a sensitive toxicologic parameter before light-microscopic changes are recognized.

Occurrence of excess smooth ER in injured liver, therefore, need not be associated with increased activity of enzymes. This fact was demonstrated in acute methyl-butter-yellow intoxication

in rats (*12*), and in patients having hepatic insufficiency from alcoholic hepatitis (*13*). Hypertrophic, hypoactive smooth ER is frequently associated with hyperplasia, or increased formation of new cells. The relation between the two phenomena needs elucidation.

FERENC HUTTERER
FENTON SCHAFFNER
FRANKLIN M. KLION, HANS POPPER
Department of Pathology, Mount Sinai School of Medicine, City University of New York, New York 10029

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Sulfadiazine-Resistant Group A Neisseria meningitidis

Abstract: *A meningitis epidemic due to Group A meningococci was unusual in that most of the strains isolated from patients were generally resistant to sulfadiazine. This is the first report of sulfonamide resistance in an epidemic strain of* Neisseria meningitidis *Group A.*

The etiologic agent of a meningitis epidemic in Meknes, Morocco, which was part of a more general epidemic involving several Moroccan cities was *Neisseria meningitidis* Group A. Such epidemics have been common in Africa, but this particular epidemic was somewhat unusual because it occurred outside the "meningitis belt," that area north of the equator and south of the Sahara (*1*). As the epidemic progressed, some of the strains isolated from cerebrospinal fluid of patients seemed to be resistant to sulfonamide drugs. This impression was subsequently confirmed. Thus, the epidemiologic situation more closely resembled that reported by Millar *et al.* (*2*), with epidemics due to sulfadiazine-resistant Group B meningococci, than the more classic epidemics caused by Group A strains. This was apparently the first meningococcal meningitis epidemic studied in which *N. meningitidis* Group A was sulfadiazine-resistant.

The Meknes epidemic lasted several months and involved several thousand cases. At its peak, approximately 20 new hospital admissions were seen each day. Of these about 75 percent were confirmed by laboratory diagnosis through culture or examination of spinal fluid. Strains of meningococci were identified by typical butyrous or viscid, grey to yellowish, colonial growth. Colonies were oxidase-positive. Typical Gram-negative diplococci were observed microscopically. Acid was produced from dextrose and maltose, and no acid was produced from levulose or sucrose. Identity was established by specific agglutination in monovalent antiserum to meningococcus.

Resistance to sulfadiazine was determined by plate-dilution technique and disc-sensitivity tests. For plate-dilution tests, measured amounts of the sodium salt of sulfadiazine were added to Mueller-Hinton agar to yield concentrations of 1, 5, 10, 20, and 40 mg per 100 ml in the medium. Petri dishes of these agars were inoculated to yield isolated colony growth. Ninety-one isolates from cerebrospinal fluid obtained during the peak period of the epidemic were tested satisfactorily by the plate-dilution technique. Disc-sensitivity tests were performed on 370 Group A meningococcal isolates obtained from the cerebrospinal fluid or blood of patients.

The sensitivity tests by both techniques revealed that many of the isolates were resistant to sulfadiazine. By the disc test, 55 percent demonstrated some degree of resistance; 38 percent were completely resistant, while 17 percent were slightly sensitive.

In the plate-dilution tests, isolates also exhibited different degrees of sulfadiazine resistance. Ninety percent of the strains were resistant to 1 mg of sulfadiazine per 100 ml. Resistance to 5 mg of sulfadiazine per 100 ml was observed in 49 percent of the isolates. Growth of 10 percent of the strains was not inhibited by sulfadiazine concentrations of 10 mg per 100 ml or greater. It has been reported that over 96 percent of Group A meningococcus cerebrospinal fluid isolates were inhibited in vitro by 0.5 mg of sulfadiazine per 100 ml (*3*). Furthermore, in tests of Group B meningococci isolated since 1963, 51 percent grew on Mueller-Hinton plates containing 5 mg of sodium sulfadiazine per 100 ml (*4*). Therefore, sulfadiazine resistance was demonstrated in many isolates from this Group A meningococcal meningitis epidemic.

Important medical and epidemiologic significance may accompany this discovery of sulfadiazine-resistant Group A meningococci. This is especially true because such strains have appeared in epidemic meningitis form. Sulfadiazine and perhaps other sulfa drugs may no longer be automatically relied on for treatment or prophylaxis of meningococcal disease. We are now faced with the same problems with Group A strains as those presented to us by the Group B sulfa-resistant meningococcus strains since 1963. For example, the carrier state cannot be eliminated from the nasopharynx of an individual by treatment with sulfadiazine when the strain encountered is resistant in vitro to more than 0.1 mg of sulfadiazine per 100 ml (*5*). Even more important, because Group A meningococci are common agents in individual meningitis cases, clinicians must now be alert to the presence of sulfa-resistant Group A meningococci in the population. Alternate choices of antibiotic drug therapy for meningitis patients should be considered.

C. E. ALEXANDER, W. R. SANBORN
G. CHERRIERE, W. H. CROCKER, JR.
P. E. EWALD, C. R. KAY
U.S. Naval Preventive Medicine Unit No. 7, Naples, Italy; U.S. Naval Medical Research Unit No. 3, Cairo, Egypt, U.A.R.; and *Laboratoire Regionale, Meknes, Morocco*

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