conditions do actually exist. For this to happen, the partitioning rate constant, k_2, must be appreciably greater than both k_1 and k_3.

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Hepatic Injury Caused by *N*-γ-Phenylpropyl-*N*-benzyloxyacetamide: A Light and Electron Microscopic Study

K. KOVACS, B. D. GARG, and J. D. KHANDEKAR*

Abstract □ W-1372 (*N*-γ-phenylpropyl-*N*-benzyloxyacetamide), a hypolipemic and antiatherosclerotic compound, given orally to rats in corn oil induces fatty degeneration of the hepatocytes. Electron microscopically, it was possible to note accumulation of lipid droplets and liposomes, progressive dilatation, disorganization and degranulation of the rough-surfaced endoplasmic reticulum, and injury of the mitochondria as well as distension of the sacs of the Golgi apparatus with disappearance of their vacuolar content. Possibly, a defect in hepatocellular lipoprotein synthesis or release might be responsible for this lipid accumulation.

Keyphrases □ *N*-γ-Phenylpropyl-*N*-benzyloxyacetamide—hepatic injury, detection by light and electron microscopic study □ Hepatic lipid accumulation—induced by W-1372, detected by electron microscopy □ Electron microscopy—detection of liver damage induced by *N*-γ-phenylpropyl-*N*-benzyloxyacetamide

N-γ-Phenylpropyl-*N*-benzyloxyacetamide (W-1372) lowers the blood level of cholesterol, phospholipids, and triglycerides (1, 2). In addition, it reduces the extent of aorta atherosclerosis in squirrel monkeys and rabbits fed on a cholesterol and fat-rich diet (1–3). The light and electron microscopic changes found in the liver of rats treated with this hypolipemic and antiatherosclerotic compound are described here.

EXPERIMENTAL

Materials and Methods—Twenty-four female ARS/Sprague-Dawley rats[1], with a mean initial body weight of 95 g. and maintained *ad libitum* on Purina laboratory chow[2] and tap water, were divided into four equal groups, one of which served as untreated controls. The second group received 1 ml. of corn oil, by stomach tube, twice daily for 3 days. The animals of Groups 3 and 4 were given 5 or 10 mg. of W-1372 (Wallace), respectively, in 1 ml. of corn oil twice daily *per os*, also for 3 days. All treated rats were killed without anesthesia, by destruction of the medulla oblongata, 16 hr. after the last gavage. The untreated animals were sacrificed at the same time.

For light microscopic examination, fresh liver tissue was fixed in alcohol formol or neutral formaldehyde and embedded in paraffin. Sections (4–8 μ thick) were cut and stained with hematoxylin–phloxine or by the Periodic Acid Schiff (PAS) technique. Frozen sections were also cut and stained with Oil red O or Sudan black B.

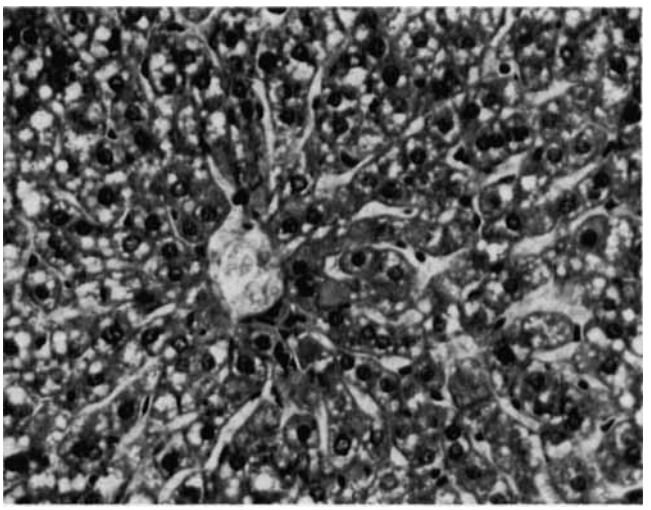

Figure 1—*Liver of a rat treated with 10 mg. of W-1372 for 3 days. Numerous lipid droplets are seen in the cytoplasm; hematoxylin–phloxine.* (×300)

Material for electron microscopic examination was obtained from three rats per group by excising a small portion of tissue from the left lateral lobe of the liver and placing it in Millonig's osmium fixative where it was minced into tiny cubes and kept for 1 hr. at 4°. The specimens were then dehydrated in graded ethanol and embedded in Epon resin. Sections (0.5 μ thick) were cut on a microtome[3], stained with toluidine blue, and examined under a light microscope. Ultrathin sections (approximately 50 nm.) were cut from selected midzonal areas, stained with uranyl acetate and Reynolds' lead citrate, and examined under an electron microscope[4].

RESULTS

The livers of rats treated with 10 mg. of W-1372 were enlarged, smooth-surfaced with somewhat rounded edges, pale brownish-yellow, and more fragile than those of the controls. A mild yellow discoloration was also noticeable after treatment with 5 mg. of W-1372, but other gross changes were not evident. No alterations were observed in the livers of animals that received corn oil alone.

[1] Madison, Wis.
[2] Ralston Purina Co. of Canada.
[3] Porter-Blum MT-2.
[4] Carl Zeiss EM 9A.

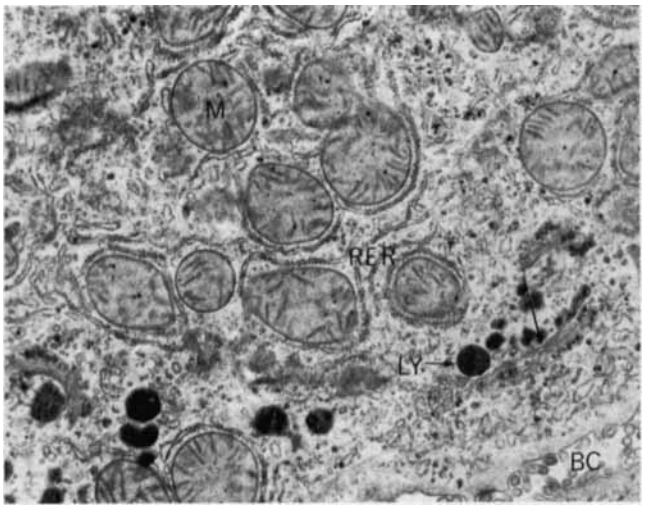

Figure 2—*Liver electron micrograph of control rat treated with corn oil alone showing characteristic features. Key: M, mitochondrion; RER, rough-surfaced endoplasmic reticulum; arrow, Golgi apparatus; LY, lysosome; and BC, bile canaliculus.* (×*13,400*)

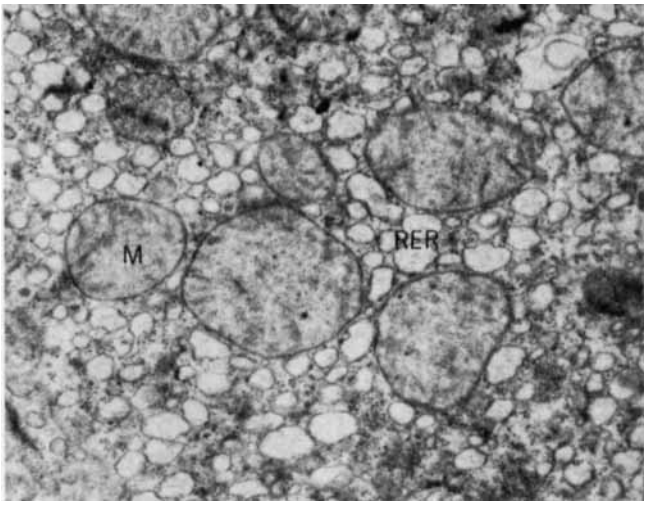

Figure 3—*Liver electron micrograph of a rat treated with 10 mg. of W-1372 for 3 days. The rough-surfaced endoplasmic reticulum (RER) is markedly distended and disorganized. The mitochondrial cristae are diminished in size and number.* (×*19,300*)

Under the light microscope, extensive lipid accumulation was seen in the hepatocytes of rats given 10 mg. of W-1372 (Fig. 1). Lipid droplets were uniformly distributed throughout the liver lobule with the exception of the hepatocytes adjacent to the central vein, which appeared to be unaffected. The droplets varied in size: in some cells, only small droplets were present; in others, they coalesced, occupied a larger portion of the cytoplasm, and displaced the nucleus eccentrically. Frozen sections stained with Oil red O or Sudan black B confirmed that the intracytoplasmic vacuoles represented lipid material.

There was a slight decrease in the amount of glycogen granules, as revealed by the PAS technique. The sinusoids were somewhat compressed by the swollen, vacuolated liver cells, but the original architecture of the liver was not distorted. Neither necrosis, inflammation, nor connective tissue proliferation was present; in experiments in which larger amounts of W-1372 were given, however, disseminated foci or necrosis was also seen[5]. Only slight lipid accumulation was found in the livers of rats treated with 5 mg. of W-1372. There were no morphologic abnormalities in animals given corn oil alone.

Under the electron microscope, many moderately electron-dense lipid droplets were seen in the cytoplasm of rats treated with 10 mg. of W-1372. These droplets varied in size and number in the individual liver cells.

The endoplasmic reticulum membranes exhibited progressive swelling, dilatation, and disorganization as compared with the controls (Figs. 2 and 3). The number of membrane-attached ribosomes was decreased. Large cisternae were formed, the walls of which were lined with dilated and partially degranulated endoplasmic reticulum membranes. Their lumina were filled with a moderately osmiophilic amorphous material. These structures corresponded to the so-called liposomes described by others (4–6). At some places, the changes were markedly advanced; the endoplasmic reticulum membranes were very irregular in shape and size, disrupted, and aggregated into almost unrecognizable masses.

The mitochondria were slightly swollen, but their double-limiting membranes were usually well preserved. The matrical density and number of intramitochondrial electron-dense granules were somewhat decreased. The cristae were diminished in size and number. Occasionally, they almost completely disappeared, endowing the intramitochondrial compartment with a homogeneous appearance.

The Golgi apparatus, which normally consists of from two to five closely parallel arrays of flattened sacs and numerous small spherical vesicles filled with a finely granular, moderately electron-dense material, also exhibited conspicuous abnormalities (Fig. 4). The sacs were markedly distended. The vesicles were dilated and, in several places, appeared empty.

[5] H. Selye and F. Lefebvre, to be published.

Milder electron microscopic changes were present in the hepatocytes of rats given 5 mg. of W-1372. No fine structural alterations were found in the livers of animals treated with corn oil alone.

DISCUSSION

Previous publications dealing with the various effects of W-1372 provide very few indications of its hepatotoxicity. Berger *et al.* (1, 2) found that this compound causes reversible liver enlargement in rats and an increase in total hepatic lipids, cholesterol, phospholipids, and triglycerides, but histopathologic abnormalities were not described.

Hepatocellular fat accumulation is the result of an imbalance between the rate of lipid synthesis and breakdown as well as uptake and elimination. In fact, it is a fundamental nonspecific reaction of the hepatocytes to various types of injury. It occurs quite commonly in man and can be induced easily in experimental animals in many ways such as: (*a*) administration of carbon tetrachloride, ethionine, ethanol, phosphorus, or puromycin; (*b*) orotic acid feeding; or (*c*) choline-deficient diets (4, 7, 8). It seems justifiable, on the basis of the present findings, to add to this list another compound that is also capable of inducing fatty degeneration of the hepatocytes: W-1372.

The structural lesions, including the electron microscopic changes found in the livers of rats treated with W-1372, are similar

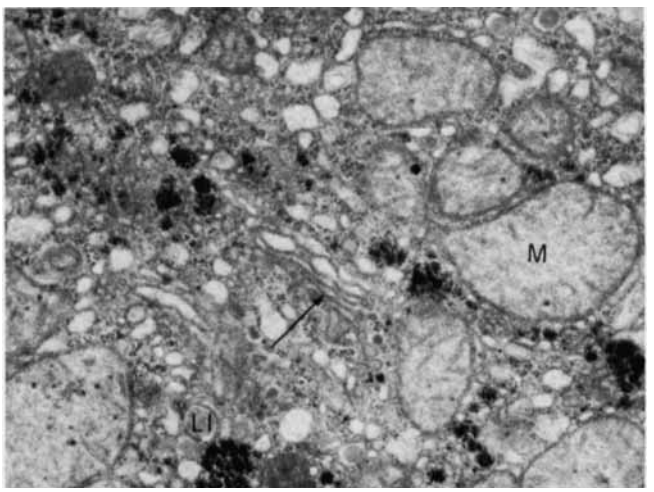

Figure 4—*Liver electron micrograph of a rat treated with 10 mg. of W-1372 for 3 days. The sacs of the Golgi apparatus (arrow) are dilated and appear to be empty. Liposomes (LI) are also seen.* (×*20,000*)

to those that occur in the course of hepatic injury such as carbon tetrachloride or ethionine poisoning (9–11). It was demonstrated that these toxicants produce fatty livers by interfering with hepatocellular lipoprotein synthesis or release (4). Furthermore, it was shown that the presence of numerous liposomes and the alterations in the Golgi apparatus, consisting of saccular dilatation and disappearance of their vacuolar content (also seen in the present experiments), are electron microscopic signs indicative of the failure of lipoprotein secretion (4, 6, 8, 12). Further investigations are, however, required to verify whether a similar mechanism is also responsible for the fatty degeneration caused by W-1372.

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* Fellow of the Medical Research Council of Canada.

Characteristics of a Highly Purified Pyrogenic Lipopolysaccharide

GAYLORD B. CASTOR, NATHAN KANTOR, EVERETT KNOLL, JOCELYN BLAKELY, JEAN K. NIELSEN, JOSEPH O. RANDOLPH, and AMIEL KIRSHBAUM

Abstract □ A highly purified lipopolysaccharide from *Klebsiella pneumoniae* was examined for qualitative chemical composition and, in a collaborative study, for pyrogenic activity. The principal constituents determined after hydrolysis were galactose and mannose, together with unidentified fatty acids. A dose of 0.001 mcg./kg. administered to groups of eight rabbits resulted in a high percentage of positive pyrogenic responses as defined by USP XVIII.

Keyphrases □ Lipopolysaccharide, pyrogens—isolation, purification, characterization, activity □ Pyrogenic lipopolysaccharide from *Klebsiella pneumoniae*—isolation, purification, and characterization, pyrogenic activity tested □ Bacterial pyrogens—isolation and purification of lipopolysaccharide from *Klebsiella pneumoniae*, determination of constituents and pyrogen activity

In a continuing effort to find a bacterial pyrogen that might be generally acceptable as a pyrogen control, scientists of the National Center for Antibiotics Analysis (NCAA) recently undertook to purify a previously known lipopolysaccharide from *Klebsiella pneumoniae* (ATCC 12833). Details of the methods used for earlier extracts of the organism were reviewed by Selzer (1).

In the latest study, a highly purified material was obtained. This paper reports on some qualitative chemical characteristics of the substance and presents the results of a collaborative study to determine its pyrogenic activity in rabbits.

EXPERIMENTAL

Isolation and Purification—The *K. pneumoniae* was maintained on slants containing 10 ml. of soybean–casein digest agar. For seeding purposes, a new slant was inoculated and incubated at 32–35° for 24 hr. With 3 ml. of sterile soybean–casein digest broth (USP XVIII), the growth was washed from the slant into a 38 × 200-mm. tube containing 100 ml. of the same broth. The broth tube was incubated at 32–35° for 24 hr. Approximately 4 ml. of the broth culture was transferred to each of 25 Roux bottles containing 300 ml. of soybean–casein digest agar with 0.25% dextrose. The bacterial suspension was spread over the surface with the aid of sterile glass beads. The Roux bottles were incubated at 32–35° for 3 days. The resulting growth was then washed from each Roux bottle with 20 ml. of sterile pyrogen-free distilled water. All washings were collected in a sterile, 1000-ml., screw-cap conical flask. One hundred milliliters of 37% formaldehyde was then added, and the mixture was allowed to stand overnight at room temperature. On the next day, the cells were centrifuged, washed with water to remove extracellular material, and freeze dried.

The basic purification procedure was that of Westphal and associates, as modified and described by Selzer (1).

Chemical Analysis—The lipid fraction of the purified material, analyzed by GC, appeared to contain several fatty acids, which cannot be identified at the present time. The carbohydrate moiety was hydrolyzed under various conditions, and the simple sugars were detected by descending paper chromatography.