145°C similarly insoluble. A small crystal of venom was placed on each of the seeded plates which were then incubated at room temperature (25°-30°C). All crystals were approximately but not exactly the same size. The results were recorded when growth was at its maximum.

Results. Of 21 organisms tested, 15 were inhibited by the hemolytic component of fire ant venom. (Table I). Results obtained with the 2 lots of crystals were similar. An example is shown in Fig. 1.

Summary. The crystalline hemolytic component of fire ant venom was tested against 21 organisms, mostly human pathogens, by placing a crystal in direct contact with Petri dishes seeded with a different organism. Antimycotic action was demonstrated by a lack of growth immediately surrounding the crystal with the following genera: *Blastomyces, Candida, Cladosporium, Cryptococcus, Geotrichum, Hormodendrum, Microsporum, Nocardia, Phialophora,* and *Trichophyton.*

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Antioxidant Effect of Chlorpromazine. (25352)

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Phenothiazine and its derivatives have been known for many years as potent antioxidants, and are used in the petroleum industry for that purpose(1). This fact suggested their trial as inhibitors of formation of lipide peroxides in biological systems. Rat brain and liver homogenates and liver mitochondria were used, and the amount of fatty acid peroxide was estimated by the thiobarbituric acid method(2). Chlorpromazine, and derivatives which are formed from it on standing in light, markedly inhibit formation of peroxides, and some properties of the reaction are described below.

Methods. Rat liver or brain was removed, iced, homogenized in 0.05 M phosphate buffer at pH 6.8, and diluted 1:10. Usually 0.4 ml of the homogenate was added to the drug solution and water to make 2 ml, and the flasks incubated aerobically at 38°. After incubation, 4 ml 20% trichloracetic acid were added, then 3 ml water, and 1 ml 0.75% thiobarbituric acid solution. The mixture was heated for 15 min in boiling water bath, centrifuged, and the red color produced by the reaction of fatty acid peroxides with thiobarbituric acid was estimated using a 520 filter. Color units are arbitrary.

Results. When solutions of chlorpromazine remained in light they rapidly darkened. These colored solutions gave no absorption peaks in the visible spectrum. The chemistry of change is not fully understood and quantitative relationship of the dark colored product to the original compound is therefore unknown. However, with the homogenized brain or liver, the antioxidant activity of the colored solution was about 5 times that of fresh material. Maximal activation occurred in a few days, and recognizable activation could also be observed in a solution kept for 24 hours at 38° in the dark. Fig. 1 shows that lipide peroxide formation in liver homogenates by both original and "activated" chlorpromazine was proportional, within limits, to their concentrations. If the concentration of chlorpromazine was kept constant and amount of liver homogenate varied, there was a linear decrease of inhibition with increasing amounts of homogenate. Thus, with 0.8, 0.4 and 0.2 ml of homogenate, 12.5 μg/ml chlorpromazine inhibited 21, 46, and 90% respectively. Percent inhibition by both chlorpromazine and its "activated" product was constant throughout the incubation period, when measured at intervals between 15 and 90 min. Thus the liver

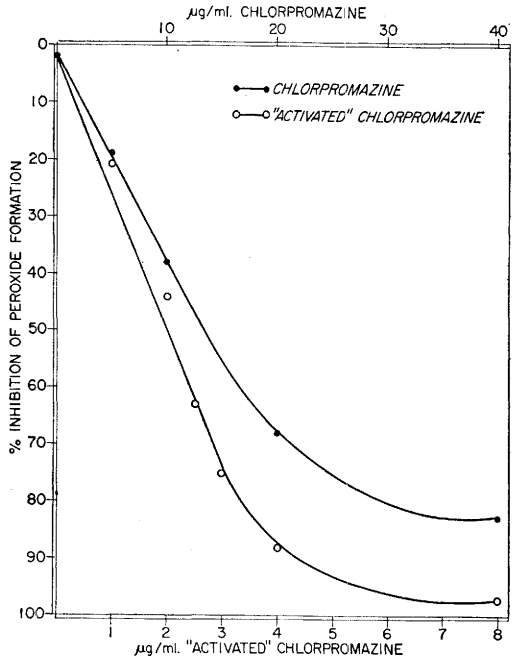

FIG. 1. Inhibition by chlorpromazine of lipide peroxide formation in rat liver homogenate incubated 60 min. at 38°; pH 6.8. "Activated" chlorpromazine was the material in solution for 4 days in the light at room temperature before use.

did not alter antioxidant activity of either compound.

Sulfoxide of chlorpromazine was 10 times less effective an inhibitor than chlorpromazine on both brain and liver. However, it too darkened on standing, and became, like chlorpromazine, 5 times more active than the original solution. Methylene Blue also inhibited production of fatty acid peroxides and on a molar basis was as active as the "activated" chlorpromazine.

When ferrous ions are added to a mitochondrial suspension, formation of lipide peroxide is much increased. A concentration of 1 x 10^{-3} M Fe^{++} increased the thiobarbituric acid color value after 1 hour incubation from 15 to 300, and this effect can be prevented if EDTA or other chelating agents are added to the system(3). Chlorpromazine, however, inhibited formation of color in very much smaller concentrations, *i.e.*, 5 x 10^{-5} M, so that chelation of only a small part of the Fe^{++} could have taken place. The chlorpromazine effect is therefore the result of some other process.

Addition of ferric iron to fresh chlorpromazine in acid causes rapid formation of a pink color, which shows maximum absorption at 530 mm and 255 mm, and fades within a few hours(4). This product was tested on liver homogenates, but was not more active than chlorpromazine.

Discussion. Although the antioxidant activity of chlorpromazine and its "activated" product is demonstrable *in vitro* in concentrations which might be present during clinical use of the drug, it is improbable that the overall pharmacological actions can be explained on this basis. However, lipides are known to be concerned with electron transport systems, and *in vitro* experiments have shown that chlorpromazine inhibits oxidation and oxidative phosphorylation in mitochondria(5). Antioxidant activity of chlorpromazine may be correlated with its ability to inhibit mitochondrial systems, in which case the "activated" form would be expected to be more effective than the fresh drug.

It does not seem likely that the antioxidant effect is related to chelation of metals; more probably, the action is connected with the tendency of phenothiazine and its derivatives to form resonance-stabilized free radicals. Their ability to inhibit lipide peroxide formation in brain and liver homogenates decreases in the following order:—Methylene Blue = "activated" chlorpromazine > chlorpromazine > "activated" chlorpromazine sulfoxide > chlorpromazine sulfoxide.

Summary. 1. Chlorpromazine, in concentrations of 2 x 10^{-5} M, inhibits formation of lipide peroxides in liver and brain homogenates. 2. When allowed to remain in light, chlorpromazine solutions become brown in color, and the activity is increased 5-fold. 3. Chlorpromazine sulfoxide is less effective than chlorpromazine, but is similarly activated on standing. 4. Methylene blue is a powerful antioxidant, comparable to the activated form of chlorpromazine.

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Digestion, Absorption and Metabolism of Chimyl Alcohol Fed as Free Alcohol or as Alkoxydiglyceride.* (25353)

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It was shown previously(1,2) that after feeding free chimyl alcohol dissolved in olive oil to rats or to human beings, the ether linkage was broken in the intestinal mucosa so that about 50% of radioactivity in lymph was associated with palmitic acid and the remainder of chimyl alcohol was present partly as chimyl alcohol and partly as esterified. This finding together with structural similarity of chimyl alcohol with a monoglyceride makes it possible to study whether a real synthesis of glycerides takes place in the intestinal contents and the forms in which dietary triglycerides are absorbed into the intestinal mucosa. In the present report the course of hydrolysis and synthesis of alkoxydiglycerides *in vivo* was followed after feeding labelled chimyl dioleate or free chimyl alcohol to rats. Furthermore metabolism and distribution of chimyl alcohol in the rat has been followed.

Methods. Labelled material: Preparation of α-(1-C^{14})-hexadecylglyceryl ether (chimyl alcohol) was carried out mainly according to Holmes *et al.*(3) as described earlier(1,2). Radioactive chimyl dioleate was prepared by esterification of α-(1-C^{14})-hexadecylglyceryl ether with oleic acid chloride in pyridine. Acid chloride was prepared from oxalyl chloride. Free fatty acids were removed by passing the mixture through Amberlite IRA-400. The neutral material was then applied in benzene to a silicic acid column and eluted with successive portions of benzene, benzene:chloroform (85:15) and chloroform. More than 95% of radioactive material was eluted with benzene. The diesterified chimyl alcohol (chimyl dioleate) thus behaved as a triglyceride. This chromatographic behavior of alkoxydiglyceride has been described(1,2). Labelled chimyl alcohol was fed as free alcohol or as chimyl dioleate dissolved in olive oil. In one series (A) intestinal contents were analyzed during absorption phase 2-4 hours after administration. In another series (B) distribution of activity in liver lipides and expired CO_2 was determined 24 hours after administration. Finally in some experiments (C) distribution of activity in lymph lipids was followed after feeding chimyl dioleate. A. Rats were fed 0.5 ml of 2% solution of chimyl dioleate or chimyl alcohol in olive oil by stomach tube. Animals were killed 2-4 hours after administration, and contents of small intestine were washed out with saline into dilute hydrochloric acid. Total fat was extracted 3 times with 2 volumes of ether. Separation of free fatty acids and glycerides of recovered lipides was performed on columns of Amberlite-IRA-400. A further separation of glycerides into tri-, di-, and monoglycerides was made on columns of silicic acid(4). In this separation chimyl dioleate followed the triglycerides, chimyl monooleate the diglycerides, and chimyl alcohol the monoglyceride fraction. B. In some experiments the same solutions as in Exp. A were fed to rats, expired CO_2 was collected and isotope content determined as described earlier(5). For comparison chimyl alcohol was injected intravenously and expired CO_2 collected. At different time intervals 24-72 hours, the rats were sacrificed and the organs analyzed. In the present paper only data on excretion of radioactivity in the breath are given. C. In a few experiments, labelled chimyl dioleate dissolved in olive oil was fed to rats with a thoracic duct fistula. The technic for collection of lymph

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