work was done, that upon reevaluation of their data they felt that these results were due, at least in part, to traumatization to the animals during injections of the drug. This leaves Grenell's(10) observation that the nucleic acids are reduced in chlorpromazine treated rats unconfirmed. It is, of course, possible that these biochemical alterations in nucleic acids are insufficient to be detected by the usual histochemical technics.

Summary. In mice receiving 15 mg/kg/day of chlorpromazine for 28 days and in rats receiving 25 mg/kg of the drug 3 times a day for 3 days, no morphological alterations in the Nissl and Golgi bodies of the cells of the cerebral cortex were noted.

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Observations on Toxins of Poisonous Fishes.* (24182)

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Certain fishes of the tropical Pacific, including fish as diverse as puffers (*Tetraodon*) and snappers (*Lutjanus*), have long been reported to be highly toxic when eaten. Hiyama(1), by feeding tests, and Halstead and Bunker(2), by intraperitoneal injections into mice of aqueous extracts of fish viscera and flesh, have confirmed these reports. This toxin is not the result of bacterial decomposition, but occurs in the flesh of fresh fish. These toxins may vary among the different species(3). The object of these tests was to explore the nature of the toxin sufficiently to permit planning of future studies on poisoning characterized by Halstead(3) as *Ciguatera*. Fish producing this syndrome are reported by islanders to be highly toxic in certain restricted areas, but elsewhere are excellent as food. To avoid any confusion of toxins that could be different, only body muscles of *Lutjanus bohar* Forskal from Palmyra were used.‡ About 20 fish were tested ranging 14 to over 24 inches long. Only fish longer than 16 inches produced the syndrome characteristic of *Ciguatera* poisoning.

Results. The first phase was to find an animal that would respond consistently with symptoms paralleling those found in man. Cats so responded, as reported by others, but were not readily available. A variety of other vertebrates was then tested, but none except the imported mongoose (*Herpestes javanicus auropunctatus*) responded to dosages, in terms of body weight, that caused paralysis and death in cats. The symptoms of the mongoose, like those of the cat, included loss of certain reflexes, for example, failure to withdraw the foot when touched lightly. A progressively severe loss of coordination followed in first the back and then the forelegs which, in acute cases, would prevent the mongoose from standing or righting itself after falling. Death, preceded by coma and

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Cheyne-Stokes breathing, would occur within 36 to 48 hours, if fish of sufficient toxicity was fed. Unlike cats and man, the mongoose never vomited, thereby permitting a more accurate assessment of the amount of fish eaten.

In tests reported below the mongoose was used exclusively; no mongoose, after once showing symptoms, was used again. Mongooses were fed raw fish to determine approximate lethal dose. No fish was found to be lethal under 5% body weight, and no fish was used that did not cause death at 15% body weight. When fluid extracts of the fish were used, they were concentrated and evaporated on Gaines dog food for feeding.

Feeding tests confirmed that toxin in fish is not noticeably reduced by drying the minced muscle at 102°-108°C for 24 hours, or by freezing the whole fish for periods up to 6 months.

Solubilities of the toxin were explored. The implication of test reported by Halstead(2,4) is that the toxin is soluble in distilled water. Ground muscle, thrice extracted with its own weight of distilled water for 24 hours, showed no loss in toxicity, but the extract, concentrated on dog food, caused no reaction when fed.

When dehydrated ground flesh was extracted with chloroform, diethyl ether, or hot absolute alcohol, neither the extract nor residue fed at twice the lethal dosage produced obvious reactions. When dried meat was extracted with 90% ethanol, all of the toxin appeared in the extract; 70% ethanol appeared to be less efficient, and absolute alcohol extracted almost no toxin. The 90% ethanol extract decreased in toxicity when it was evaporated to dryness without dog food.

When secondary extraction of the 90% ethanol solution was made with repurified ether, dioxane, chloroform or petrol ether, no observable toxicity was left in either fraction. However, under an atmosphere of commercial nitrogen (about 3% oxygen) toxicity of extracts was diminished but not lost. When the alcoholic solution was evaporated to dryness under nitrogen, then extracted with petrol ether and the undissolved residue redissolved in 90% ethanol, both fractions gave toxic symptoms. When the experiment was repeated with chloroform or diethyl ether, toxicity was observed only in the chloroform and diethyl ether solutions.

The reliability of Halstead's technics(2,4) was also investigated. Halstead homogenized portions of fish, *i.e.* muscles, viscera, etc., in water, centrifuged the slurry for 25 minutes at 2,000 rpm, and injected 1 ml of supernatant fluid intraperitoneally in 15 to 25 g mice.

Halstead recognized 3 types of mouse reactions to poisonous fish: *weakly positive,* including lacrimation, diarrhea, and ruffling of hair, but the animal recovered; *moderately positive,* including same symptoms, but the animal died within 36 hours; and *strongly positive,* where test animal died within one hour.

Mice showed no symptoms when fed toxic fish even at twice the dose in percentage of body weight that was lethal to a mongoose. An extract was prepared according to the method of Halstead and Bunker(2,4) of a lethally toxic fish and of a non-toxic tuna; these extracts were injected into mice weighing from 20 to 25 g. The 2 mice receiving the tuna injection showed no reaction, but the 2 receiving the lethal fish displayed what Halstead called a "weakly positive" reaction, with slight lacrimation, diarrhea, and ruffled fur.

The extract prepared by this technic was not a solution but a milky suspension. Since mice injected with the most toxic *Lutjanus* available did not die, a fish classified by Halstead as having a different type of toxin was used to determine whether the toxin was in solution or suspension. Liver and gonads of the puffer [*Arothron hispidus* (L.)] were prepared by Halstead's technic; after centrifugation the extract was divided into 2 portions. One portion was filtered twice through filtered paper and finally through a Millipore glass filter. Two mice, weighing between 20 and 25 g, were each injected intraperitoneally with one ml of this solution, while the unfiltered extract was injected into 2 similar mice. The 2 mice receiving the *unfiltered* extract

died but the ones injected with the filtered extract showed no symptoms.

Summary. 1. Not all animals are sensitive to the *Ciguatera*-type toxin. 2. This toxin is not water soluble, but is soluble in 90% ethanol and certain other solvents. 3. The toxin may be detoxified when concentrated in normal atmospheric oxygen, but remains toxic under nitrogen. 4. The test previously used to assess this toxicity is not sufficiently sensitive and depends upon the suspended matter in the extract.

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Ability of Human Plasma to Lyse Homologous Erythrocytes Pretreated with Fatty Acid. (24183)

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Addition of washed mammalian erythrocytes to fatty acid solutions results in hemolysis, the rate dependent upon fatty acid and erythrocyte concentration and upon pH and temperature(1). Addition of homologous plasma or plasma protein fractions to fatty acid *prior* to insertion of washed erythrocytes has long been known to retard or inhibit hemolysis(2-4). This inhibiting effect has been attributed to binding of fatty acid by plasma constituents, either in solution or at surface of red blood cell(5). To date, we are unaware of any data indicating action of homologous plasma or plasma protein fractions *after* erythrocyte contact with fatty acid. This report describes ability of human plasma to lyse homologous erythrocytes pretreated with fatty acid.

Materials and methods. All fatty acid solutions were prepared in sodium phosphate buffered saline, ionic strength 0.15, pH 7.2. Normal human erythrocytes of all major blood groups were tested. Erythrocytes were washed 3 times in buffered isotonic saline and resuspended in a concentration such that 0.5 ml added to 2.5 ml of fatty acid solution resulted in 5 x 10^5 cells/cu. mm. Human plasma protein fractions were prepared by dialysis of plasma against ammonium sulfate at 4°C. The globulin-rich fractions were precipitated at 50% ammonium sulfate saturation and the residual albumin-rich fractions at 100% saturation. All precipitates were washed with appropriate ammonium sulfate solutions, resuspended in buffered isotonic saline, and dialyzed at 4°C against isotonic saline until free of ammonium ion (verified with Nessler's reagent). The final volume of plasma fractions was adjusted with buffered isotonic saline (pH 7.4) to equal that of original plasma volume. Human erythrocytes were added to varying concentrations of sodium oleate at 25°C for 3 minutes. The red blood cells were then spun at 3000 rpm, 0°C for 3 minutes and washed 5 times with 5 ml aliquots of iced buffered saline. Following the last washing, the packed red blood cells were rewarmed to 25°C and 1 ml of homologous plasma or homologous plasma protein fraction added with gentle agitation. After 2 minutes at 25°C, erythrocyte suspensions were spun at 3000 rpm, 0°C for 3 minutes and resulting hemolysis determined with the Klett-Summerson photoelectric colorimeter (filter 550 mμ).

Results. Over one hundred tests were performed with varying concentrations of fatty acid. Typical results are given in Table I.