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.

TABLE I. Ability of Human Plasma and Plasma Fractions to Lyse Homologous Erythrocytes Pretreated with Fatty Acid.

Solution added to packed human erythrocytes washed 5 times after pretreatment with oleate (1.0 ml)	% hemolysis 5 min. after addition of plasma or plasma fractions* Pretreatment oleate conc.: 1×10^{-4} M	$.3 \times 10^{-4}$ M
Isotonic buffered saline (control)	15	0
Homologous plasma	48	0
Dialyzed homologous plasma	50	0
Homologous plasma diluted 1:16 with isotonic saline	48	0
Homologous albumin fraction	100	100
Homologous globulin "	100	100
Homologous albumin + globulin fractions, mixed 1:1	100	100
Homologous plasma saturated with sodium oleate†	17	0
Homologous albumin fraction saturated with sodium oleate†	18	0
Homologous globulin fraction saturated with sodium oleate†	17	0

* Mean of 4 determinations on a single batch of plasma and erythrocytes. Per cent hemolysis determined photometrically on supernates prepared by centrifugation at 3000 r.p.m., 0°C for 3 min.

† Oleate binding saturation considered attained with maximum quantity of added oleate failing to lyse washed human erythrocytes at 37°C in 3 hr.

The findings indicate:

A) Human plasma or albumin or globulin fractions hemolyze washed homologous erythrocytes pretreated with sodium oleate. Similar results were obtained with heparinized, oxalated, or citrated plasma, and with serum.

B) Lytic activity of homologous plasma albumin and globulin fractions for oleate pretreated erythrocytes is more intense than that of whole plasma. This cannot be ascribed to dialysis or to protein losses in preparing albumin and globulin fractions since dialysis or 16 fold dilutions of whole plasma does not alter the results. Rather the findings suggest the presence of a lytic inhibitor in whole plasma which can be removed or inactivated by ammonium sulfate fractionation. Studies now in progress suggest that this inhibitor is plasma unesterified fatty acid.

C) Saturation of oleate binding capacity of human plasma or plasma protein fractions by pretreatment with exogenous oleate inhibits the lytic activity of plasma for the oleate pretreated erythrocytes. These findings suggest that human plasma and plasma albumin or globulin fractions lyse homologous erythrocytes by combining with oleate molecules fixed to the surface of the red blood cell. Whether plasma acts by "tearing" the oleate molecules from the cell membrane or by forming a new surface complex is as yet undetermined. Failure to prevent the lytic reaction by repeated washings of the oleate pretreated erythrocytes indicates the firmness of the oleate-erythrocyte bond.

Concentration of sodium oleate utilized to pretreat human erythrocytes was an important determinant of lysis following exposure to homologous plasma. Human erythrocytes exposed to oleate at concentrations which, after repeated washings, fail to cause hemolysis faster than that of untreated red blood cells, were not lysed by homologous plasma or plasma fractions. Human plasma and plasma albumin and globulin fractions thus appear to act upon homologous erythrocytes pretreated with oleate by accelerating oleate hemolysis. This does not necessarily imply that the accelerated lysis occurs *via* the same intermediate steps as the control oleate lysis.

The present findings are not restricted to oleate or to human erythrocytes. The other fatty acids tested, stearate and palmitate, yielded hemolytic reactions with homologous human plasma qualitatively similar to those of oleate. Moreover, plasma of rats, guinea pigs, sheep, and rabbits were also found capable of accelerating lysis of homologous erythrocytes pretreated with fatty acid.

Summary. Addition of human plasma to washed homologous erythrocytes pretreated with sodium oleate results in hemolysis. Whole plasma is a less efficient lytic agent than is the homologous albumin or globulin moieties prepared by ammonium sulfate fractionation. Saturation of fatty acid binding capacity of plasma or plasma protein fractions, by addition of exogenous oleate inhibits lytic activity. Hemolysis appears related to acceleration of lytic activity of oleate molecules fixed to the surface of the red blood cell following binding by plasma constituents. Similar results were obtained with other fatty

acids and with erythrocyte-plasma systems from other mammalian species.

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Deiodination of Thyroxine and Other Iodinated Compounds During Desalting by Electrodialysis.* (24184)

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Paper chromatography of thyroxine and its derivatives in a Ringer solution used for tissue metabolism studies is often unsatisfactory because the salts lead to training of the spots. Since desalting of solutions of ordinary amino acid can easily be accomplished using the electrolytic desalter of Consden, Gordon and Martin(1), an apparatus based on their design (Research Equipment Co.) was tried. The present study of rapid deiodination confirms and considerably extends the alterations in some iodinated compounds reported by Smith *et al.*(2) and by Jepson and Smith(3).

Methods. The desalter achieves results by electrolytic reduction of cations which are amalgamated at the cathode and passage of anions through a cellophane membrane into a flushing electrolyte solution. Conditions were chosen to remove completely the Na^+ and Cl^- from an isotonic NaCl solution, starting with 10 volts D.C. and 5-12 amps. The operation was finished in 10-15 minutes, as indicated by decrease in current to 0.2 amp. About 1 to 2 mg of iodinated substance† were dissolved in 0.05 ml N NaOH and diluted to 4 ml with 0.9% NaCl. Of this solution, 3.5 ml were treated in the chamber of the desalter. Aliquots representing before and after deionization were subjected to ascending chromatography, using n-butanol, ammonium hydroxide, water (250:72:178); n-butanol, glacial acetic acid, water (78:5:17); or methanol and 0.2 M ammonium acetate (100:250). The amino acids were revealed with ninhydrin(4, p. 88), phenolic substances with the Pauly reagent(4, p. 253) and iodine-containing compounds with ceric sulfate-arsenious acid(5).

Results. Thyronine, thyrosine and phenylalanine solutions all were desalted without loss of amino acids. The R_F values are shown in Table I. In contrast, mono- and diiodotyrosine were rapidly deiodinated to tyrosine. 3,3'-Diiodothyronine, 3,5,3'-triiodothyronine and thyroxine were deiodinated to thyronine, although in the last case, some free iodide remained in the cathode compartment. The iodine was also completely removed from 3,5-diiodothyronine, but some additional change was produced, since the product was clearly not thyronine. Further identification has not been possible, beyond demonstrating (Table I) that the material is a single phenolic amino acid (Pauly and ninhydrin-positive) not containing iodine. From the intensity of the ninhydrin and Pauly stains, it is probable that there was no loss of amino acid itself.

Table II shows the results of desalting car-

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