do not disprove this idea, they do suggest that it needlessly confuses the matter, since a general explanation in terms of different amounts of Pfr is consistent with all available evidence (*10*).

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Thalidomide Solutions

In a report in *Science*, entitled "Intravenous injection of thalidomide in pregnant rabbits" by Fox *et al.* (*1*), the authors claimed to have produced a supersaturated solution of thalidomide (1 mg/ml) in a medium containing carboxymethylcellulose (CMC, 5 mg/ml) and glucose (50 mg/ml). In brief, their preparation was as follows: 0.5 g of CMC was heated with 100 ml of water until the mixture became a clear solution (about 10 minutes). Five grams of glucose was dissolved in the solution, and 100 mg of thalidomide was added. The mixture was then boiled until all of the thalidomide was dissolved (about 20 minutes). The solution was immediately filtered, cooled in a water bath to 50°C, and then infused, at a temperature of about 40°C, into pregnant rabbits.

Since thalidomide undergoes spontaneous hydrolysis in neutral and alkaline solutions even at room temperatures (*2*), the drug might be rapidly converted to its hydrolytic products under the conditions described by the authors. Using their method, we prepared solutions of tritiated thalidomide and then assayed them for thalidomide by a method developed in our laboratory. A portion of the solution was shaken with ten volumes of ethylene dichloride (EDC). Over 90 percent of the thalidomide, but less than 2 percent of the hydrolytic products, was extracted into the organic phase. Part of the extract was assayed for radioactivity in a liquid-scintillation spectrometer; another was subjected to radio-chromatography. Our results indicated that the amount of thalidomide in the solution after it cooled to 40°C ranged from 4.5 to 30 percent of the amount added. The variability depended on the vigorousness of boiling; intense boiling caused more hydrolysis than slow boiling did.

The possibility that tritiated thalidomide is hydrolyzed more rapidly than the unlabeled compound does not seem likely, since virtually identical values for the amounts of unchanged thalidomide were obtained when the EDC extracts were assayed by both the radiochemical method and that of ultra violet spectroscopy of Green and Benson (*3*).

Boiling the solution of CMC and glucose with thalidomide caused the *p*H to drop from an initial value of about 7.1 to a value of about 5.3, as the acidic metabolites were formed. Since hydrolysis of the drug proceeds more rapidly in neutral than in slightly acidic solutions, the presence of buffers would obviously influence the rate of hydrolysis. Carboxymethyl cellulose might have served as a weak buffer in the authors' medium, for this substance contains from 7 to 8.5 percent sodium (*4*). Indeed, when 100 ml of distilled water (*p*H 6.82) was boiled with 100 mg of thalidomide, only about 10 percent of the drug was hydrolyzed. Since different batches of CMC might be expected to have different buffering capacities, we also tested a sample of the batch of CMC used by Fox and his co-workers. We found that from 70 to 95 percent of the thalidomide was hydrolyzed by the solubilizing technique.

Because the degree of hydrolysis which we have found in the preparation of thalidomide solutions is variable, the amount of thalidomide Fox and his co-workers administered to the pregnant rabbits cannot be determined. However, our results indicate that it probably was not greater than 30 percent of the stated values. Hence, their failure to observe teratogenic effects in rabbits after intravenously administering the thalidomide solution might be partially caused by inadequate dosage.

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We do not disagree with the guarded conclusion put forth by Schumacher, Blake, and Gillette and regret that we did not pay more attention to the hydrolizing effect of carboxymethylcellulose (CMC) on thalidomide at the start of our experiments.

We also agree that the method of preparing the solutions gives various results in the hands of different experimentors, variations mainly caused by the vigorousness of the boiling. Using the same batch of CMC we sent to Schumacher *et al.* and the same extraction method (ethylene dichloride) followed by determination of the thalidomide content of the residue with the method of Green and Benson, we obtained at the laboratory of Sloan-Kettering Institute values of 35 to 55 (compared to 5 to 30 percent obtained by Schumacher *et al.*).

The intravenous injection of thalidomide to pregnant rabbits plays an important part in the thalidomide problem. We have started a new series of experiments eliminating CMC and shall report the results in due time.

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2 September 1966