by the same way as described the above experiment. And the others, IV and V, in Table I were prepared by the same method.

Test of *in Vitro* Bacteriostatic Activity——Bacteriostatic activity was tested against *Staphylococcus aureus* 209 P in a modified Kuwabara's medium[5] without choline, base components of nucleic acids, folic acid, biotin, riboflavin, calcium pantothenate, pyridoxine, Mg^{2+} and Ca^{2+}. [6] The minium bacteriostatic concentration was estimated from the turbidity of the test solution incubated for 24 hr and 37°.

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Complications in Using Rabbits for the Study of Oral Drug Absorption

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In the past, rabbits have often been used by us[2,3] and others[4–13] as a tool in the evaluation of oral absorption characteristics of drugs because of their low cost and ease of handling. However, whether the results obtained from rabbit study will truly reflect the absorption characteristics of drugs from the human gastrointestinal tract have not been reported. It is the purpose of this communication to explore the complications and shortcomings of using rabbits for oral absorption studies.

Experimental

New Zealand white rabbits weighing from 2.0 to 2.5 kg were given 30 ml of a 25% barium sulfate aqueous suspension through a stomach tube. Anteroposterior radiographs of the abdomen of fasted and unfasted rabbits taken in the upright position were obtained at different times. The stomachs of normal and fasted rabbits were also opened and examined.

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Results and Discussion

In order to avoid the possible interactions between various foods and drugs and the effects of food on stomach emptying time in the study of drug absorption, it is a common practice to administer drugs to fasted human subjects or experimental animals. In humans, the stomach will usually be empty after several hours or certainly after overnight fasting. Therefore, drugs are usually given to human subjects in the morning before breakfast. However, when stomachs of unfasted rabbits were examined they were always found to be full of solid food. Considering rabbits are nocturnal animals this might be anticipated. Even after fasting the animal for 24 hours, the stomach was almost as full as that of unfasted rabbits, but the contents were less solid in consistency. To reduce coprophagy as much as possible, the animals were maintained on a wire mesh support which allowed feces to drop to the bottom of the cage where it was inaccessible. In spite of this precaution, even after one week of fasting with free access to water containing 5% glucose, the stomach still contained a significant degree of debris and solid material. Figures 1-A, 1-B and 1-C are X-ray photographs after the administration of a 25% barium–sulfate aqueous suspension to a rabbit whose food was withdrawn 24 hours prior to the study and maintained on water–glucose throughout the study. Note that barium sulfate still remained in the stomach and through the intestinal tract even after seven days of fasting (Fig. 1-C).

As shown in figures 2-A, 2-B, and 2-C, the rate of stomach emptying of barium sulfate in the unfasted state is much faster than in the fasted state. After six hours of administration, most of the barium sulfate has moved to the small intestine and colon. Barium sulfate could not be detected in the stomach 24 hours after administration.

Shunichi Naito[13] used rabbits to study the influences of several additives on the absorption of aminopyrine and concluded that urea, mephenesin and barbital could enhance its absorption. He fasted the rabbits overnight and withheld the food during the experiment. Since

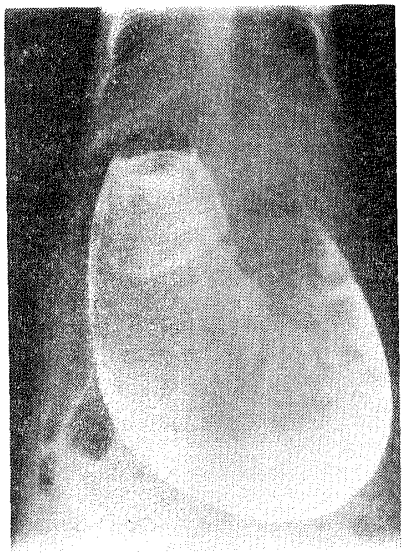

Fig. 1-A. Film taken Immediately after Ingestion of 30 ml of a 25% Barium Sulfate–H_2O Suspension through a Gastric Tube

Animal fasted 24 hours before administration. Note gas, barium and food in the stomach. White, clear areas due to barium.

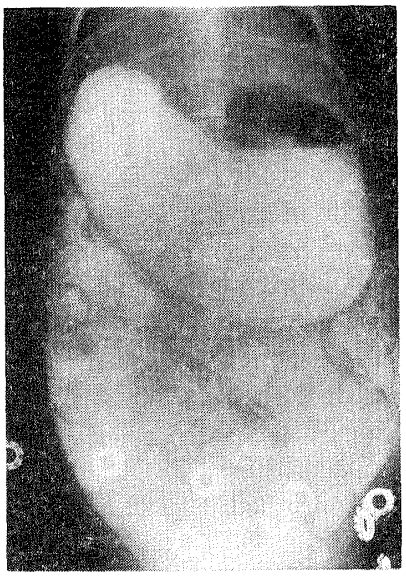

Fig. 1-B. Repeat Film at Two Days in Fasted Rabbit

Barium sulfate has mixed with stomach contents. Some barium has passed through into the small bowel and colon.

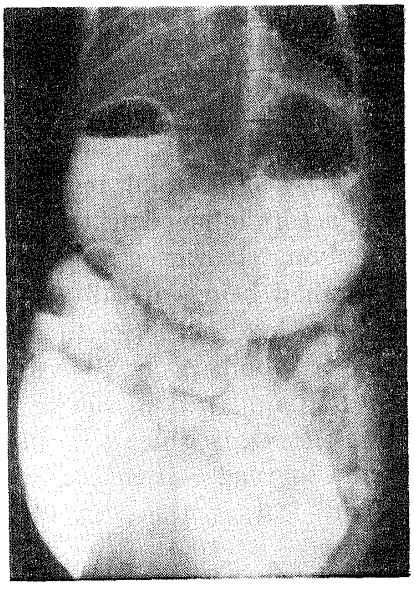

Fig. 1-C. Repeat Film at Seven Days in Fasted Rabbit

Very little change is apparent.

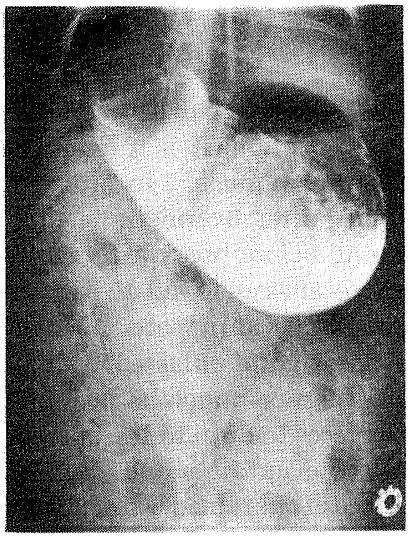

Fig. 2-A. Film taken Immediately after Ingestion of 30 ml of a 25% Barium Sulfate–H_2O Suspension

Animal had not been fasted. Note gas, barium and food in the stomach.

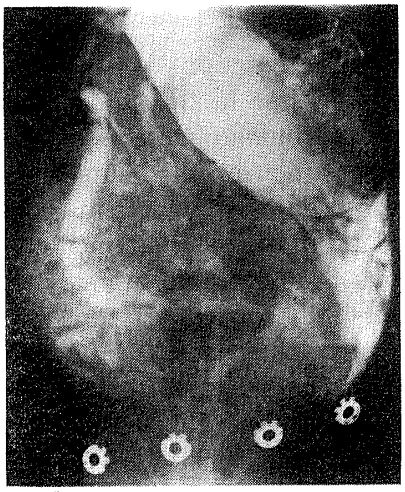

Fig. 2-B. Repeat Film 2 Hours Later in Unfasted Rabbit

Barium sulfate is in the small bowel. There is still a large amount of barium present within the stomach.

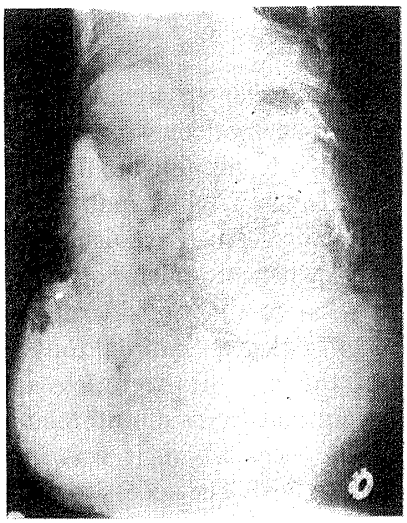

Fig. 2-C. Repeat Film 6 Hours Later in Unfasted Rabbit

Small amount of barium sulfate remains in the stomach. Most of the barium is in the small bowel and colon. Note the radiopaque scybala in the colon.

the aminopyrine is given in the state of solution and will not precipitate in the stomach fluids, it should present no absorption problem. Aminopyrine has been shown by Schanker[14] and Hogben[15] and their coworkers not to be absorbed from the stomach in man and rats. It is likely, therefore, that the drug solution administered by Naito had to transfer into the intestines in order to facilitate absorption. The significantly increased absorption observed by Naito after concomitant administration of the additives may be due to an increased rate of stomach emptying induced by the additives.

The absorption of micronized griseofulvin in both humans and rabbits has been studied in this laboratory and elsewhere.[2–5,16–18] It was proposed that the rabbit was a useful test animal to study the factors influencing the absorption of griseofulvin. However, the blood level peaks in human beings usually occurred approximately 2 to 4 hours after administration, while that of rabbits occurred six to eleven hours after administration. Peak levels of 2 to 4 hours for rats,[19] cats,[20] and dogs[21] have been observed. If rabbits were fasted after administration, the peak plateau continued

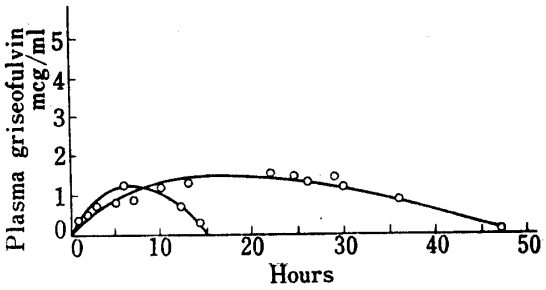

Fig. 3. Blood Levels of Rabbit 14 after Administration of Griseofulvin 150 mg/kg (Ref. 1).

open circles: normal food and water permitted during the experiment
closed circles: only water permitted after administration of the drug

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for more than twenty hours (Fig. 3). This unusual phenomenon can be explained by the fact that the stomachs of rabbits emptied very slowly in the fasted state. Since a large amount of this water insoluble drug was given, the stomach acted as a reservoir and released the drug to the intestine, presumably the main absorption site, over a long period of time.

Recently a deconvolution method[22,23] has been proposed to study the *in vivo* dissolution rate of drugs. By this method, the dissolution rate of drugs *in vivo* is compared directly with that given in the solution. Owing to the fact that the rabbit stomach is always full of solid material and that its emptying time is a function of the fasted or unfasted state, the absorption of drugs in rabbits will be greatly influenced not only by the rate of drug dissolution, but also by possible drug–food interactions and by their effects on stomach-emptying time.

From the facts presented above that it is almost impossible to obtain an empty stomach in rabbits by using the conventional method of fasting and the fasted state markedly prolongs the stomach emptying time, it is, therefore, concluded that the rabbit is not a useful animal in which to study drug absorption.

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Spectroscopic Studies on Molecular Interactions. III.[1] Improvement of the Benesi-Hildebrand Method for the Determination of Equilibrium Constants[2]

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There are numerous literatures on the properties of weak complexes such as charge–transfer complexes,[4] and the determination of equilibrium constants for 1:1 complexation is often made by the spectroscopic method proposed by Benesi and Hildebrand[5] (B–H) or some equivalent[6] of it. The B–H method can be employed to the systems where the initial concentration of the one component is in great excess of that of the other.[5] But Drago and Rose[7] indicated the inaccuracy of the complexation constant obtained by applying the B–H equation to the triethylamine–iodine system[8] where the initial concentration of triethylamine

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