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Toxicity Studies on Rutin.

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Rutin is a flavonol glycoside that has been found clinically to have a beneficial effect in a variety of conditions characterized by abnormal fragility of the capillaries.[1-4] Its action is thus similar to that of vitamin P, the term applied by Rusznyák and Szent-Györgyi[5] to a preparation which they believed led to a decrease in capillary fragility. The substances which have been reported to possess this activity have been flavonol derivatives or compounds closely related to the flavonols,[1,6] and of these rutin (quercetin rhamnoglucoside) is found in a number of common plants. The preparation of rutin from buckwheat is economically feasible.[7]

Toxicity data on the flavonol glycosides are scanty and knowledge of chronic toxicity is especially limited. Since clinical experience indicates that treatment, to be effective, must be continued for long periods, chronic toxicity studies are especially important. Garino[8] administered rutin, quercitrin, hesperidin and naringin by mouth or parenterally into dogs; some of these compounds were eliminated in the urine unchanged, and none was found to be toxic. Czimmer[9] reported, without details, that a quercetin glycoside obtained from forsythia could be administered parenterally in relatively large doses for a long period of time to a variety of animals without eliciting any symptoms. (This glycoside from forsythia has been identified by Couch, Naghski and Porter[10] as rutin). von Jeney, Vályi-Nagy, Váczi and Mihalik[11] injected 10 mg/kg of quercitrin into dogs without untoward effects, although blood lactic acid values were lowered. Wilson and DeEds[12] fed diets containing up to 1% of the flavonone glycosides, naringin and hesperidin, to rats for 200 days. Growth and organ weights of the animals were not affected and the tissues of hesperidin-treated animals, which were examined microscopically, were normal. Giltner[13] has fed rutin to albino guinea pigs and exposed the animals to light; these animals did not show signs of light sensitization such as occurs in animals eating buckwheat, an important point, since the principal source of rutin at the present time is buckwheat. Clinical reports by Griffith, Couch, Lindauer[2] and by Shanno[3] indicate that a daily oral intake of 40 to 120 mg of rutin for periods up to 9 months can be taken without the patients showing any signs of toxicity from the drug, and Griffith, Lindauer, Shanno and Couch, in their exhibit at the meeting of the American Medical Association in 1946, stated that in some cases the time had been extended to 36 months.

Experimental. Acute toxicity. In the

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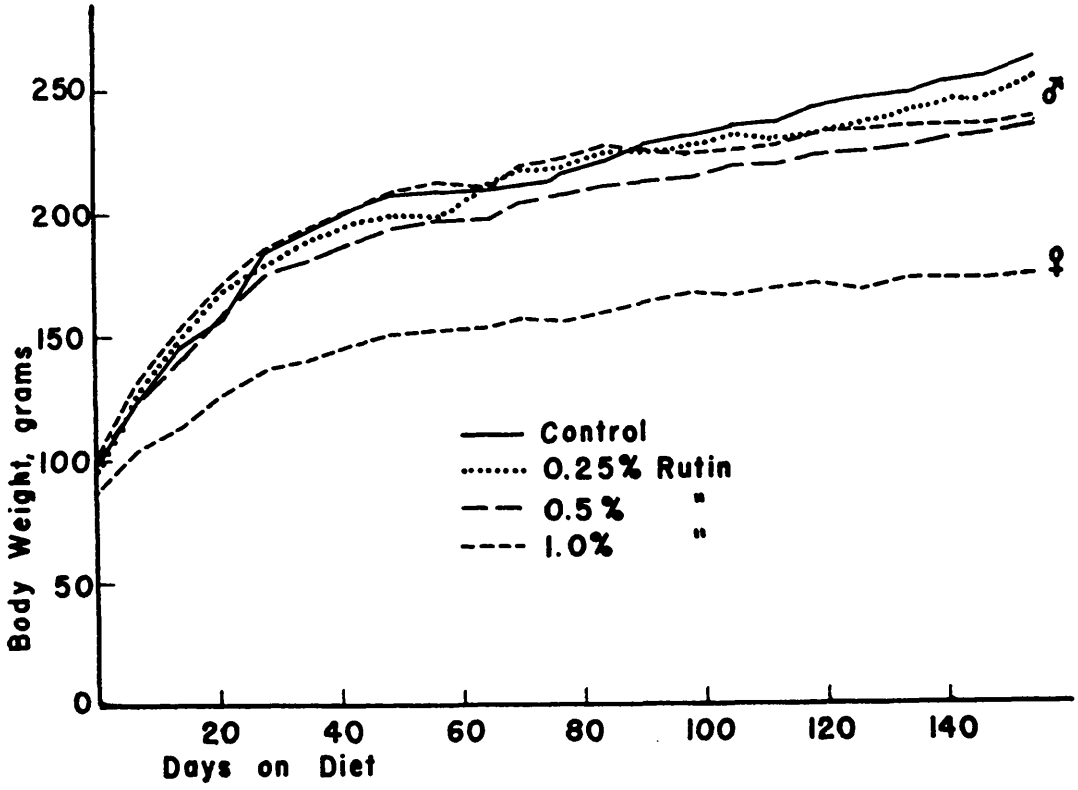

FIG. 1.
Growth curves of rats on Rutin-containing diets.

course of studies to be published later on the pharmacology of rutin,* certain data have accumulated on the acute toxicity of the substance. Adult rats and guinea pigs weighing 200 to 350 g have been injected with solutions containing 10 mg/ml of rutin. Ordinarily, the solvent was 5% ethyl alcohol, the rutin being soluble in the hot solvent and precipitating out slowly enough on cooling so that injections could be made. Occasionally, Ringer's solution was used as the solvent; rutin is fairly soluble in Ringer's, but a darkening of the solution indicated some chemical change and, for this reason, such a solution was seldom used. Sixteen rats were given 10 mg per animal, or approximately 50 mg/kg, intraperitoneally, and 8 guinea pigs 10 mg per animal, about 30 to 40 mg/kg, intraperitoneally or intravenously. Twelve rabbits were injected intravenously with a 20% solution of rutin dissolved in propylene glycol; the dosage was 100 or 200 mg/kg. No symptoms were observed in any of these animals.

Chronic toxicity. Albino rats were used for the studies of chronic toxicity. These animals, mostly males and weighing about 90 g at the start of the experiment, were divided into groups of 6 animals each and fed diets containing from 0.25 to 1.0% of rutin thoroughly mixed into finely ground Purina Dog Chow.† Growth curves and food intakes were recorded for 150 days (Fig. 1). Growth was essentially normal at all levels of rutin. The female animals receiving the 1.0% diet had no controls. These animals were used later in connection with studies on reproduction. Their growth curve is in-

* The rutin was furnished us by the Eastern Regional Research Laboratory of the U. S. Department of Agriculture, Philadelphia.

† "Purina Dog Chow" is named as part of the specification of the exact experimental conditions, and it is not implied that this product is better than or inferior to any other commercially prepared dog feed.

cluded on the chart to indicate that administration of the diet was for the same period as for the males on the same diet. At the end of this period, the control rats and those on the 0.25 and 0.5% rutin diets were autopsied and the liver, spleen, kidneys, adrenals, testes and heart were weighed. The weights of the organs did not deviate from control values to a statistically significant degree, and the gross appearance of all tissues was normal.

The 6 males and 6 females on the 1% rutin diet were continued on the diet following completion of the growth records. They were mated, with results recorded below, and afterwards observed periodically until they had been eating the diet for 400 days. At autopsy, the gross appearance of the tissues was normal; organ weights were not obtained.

Tissues from all of the animals (both the 150-day and 400-day rats) were embedded in paraffin and sections were stained with hematoxylin and eosin. Histopathological study of these sections was made by Dr. A. J. Cox, Jr., Stanford University School of Medicine, San Francisco, who reported that there were no striking changes in any of the rats as compared with the controls. The animals which received 1% rutin in the diet for 400 days showed slight irregularity in vacuolation of the cortical cells of the adrenals, which may signify a little irregularity in lipoid content of the cells. However, this was not pronounced and is of doubtful significance. In 3 of the 6 female rats which received the 1% rutin diet there was distinctly more brown granular pigment in the renal epithelial cells than in the controls. However, since all of the control animals were males, this observation cannot be interpreted. The male rats receiving the same amount of rutin did not show this change.

It was mentioned above that at the end of 150 days, the animals on the 1% rutin diet were used for observations on reproduction. There were 6 pairs, caged together for a month. From these unions there resulted 4 litters from 3 of the females (one of which must have been impregnated immediately after the birth of the first litter). One of the 4 litters was born dead and the other 3 were small (3 to 5 per litter). Since the average for the colony is considerably better than this, the experiment was repeated with variations using more animals.

At 3 months of age, 30 healthy female rats and 15 males were placed on their respective diets—Purina Dog Chow containing 1% of rutin for the experimental animals, and the chow alone for the controls. Fifteen females and all of the males ate the rutin diet. After a month the animals were divided into 15 groups, each group containing a male, a female on the rutin diet, and a control female which was a litter-mate of the experimental female. To keep the experimental animals from eating control diet, and the control rats from ingesting rutin, food was withheld during the periods when the animals of a group were caged together. These periods were limited to about 7 hours, and there were 18 of them in the month of the experiment. Admittedly such excessive handling would not be conducive to good breeding, but chances for impregnation should be equal for control and experimental rats. As a result of these matings there were 4 litters born to the females eating the rutin diet, and 2 litters to control females. The litters varied considerably in size, mortality was not extreme. It is concluded that 1% of rutin in the diet did not limit the fertility of these animals.

Several of these litters were allowed to grow until weaning. The young of the mothers on the rutin diet were as large and active as those of the control females.

As a further check on the influence of rutin on reproduction, estrus studies were made for a 4-week period on 6 female rats eating a 1% rutin diet and 6 control females. The length of the estrous cycle was not modified by the ingestion of rutin.

Discussion. Clinical use of rutin, as noted earlier, can be expected to be for long periods of time, as present indications are that discontinuance leads to a recurrence of the original condition. For this reason, the most important part of a toxicity study of rutin must be the effect of chronic ingestion. In-

gestion by rats of a diet containing 1% of rutin for 400 days amounts, in the adult animal, to about 0.6 g/kg/day, and 400 days is well over one-third of a normal rat life. Such an intake is far in excess of any dosage likely to be administered for therapeutic purposes.

Conclusion and Summary. Rutin, a flavonol glycoside with vitamin P activity, was studied for signs of acute and chronic toxicity. In rats and guinea pigs, intravenous and intraperitoneal injections of 30 to 50 mg/kg, and intravenous injections in rabbits of 100 to 200 mg/kg, were inocuous. The rate of growth of albino rats was not affected when the diet contained as much as 1% of rutin, and after 400 days on such a diet, histological examination of the tissues showed no evidence of injury which could be definitely related to rutin administration. Organ weights of the experimental animals were normal. The length of the estrous cycle was the same in rats eating a 1% rutin diet as in control animals, reproduction was as good, and the young appeared to be as healthy. On the basis of the criteria employed, rutin is nontoxic both acutely and chronically.

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Two Antibiotics (Lavendulin and Actinorubin) Produced by Two Strains of Actinomyces. III. Toxicity and Therapeutic Studies.*

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Two new antibiotics, lavendulin and actinorubin, produced by 2 strains of Actinomyces isolated from soil have been described.[1,2] The active substances have been purified and isolated and found to be of a basic nature.[3] It is the purpose of this publication to describe the toxicity and therapeutic studies which were carried out in mice.

Materials. Test animal. White mice weighing 17-19 g were used throughout the experiments.

Antibiotics. Partially purified preparations[3] of lavendulin and actinorubin were used. The purest preparation of lavendulin used contained 4.5% ash. By the agar plate method of assay[3] 0.65 μg per ml of nutrient agar inhibited the growth of *Escherichia coli.* By the method of assay in which serial dilutions of the material are made in nutrient broth,[2] 0.078 μg per ml of broth inhibited the growth of *E. coli,* P216. The purest preparation of actinorubin used contained 2.8% ash. By the agar plate method of assay,[3] 0.55 μg per ml of nutrient agar inhibited the growth of *E. coli.* By the method of assay in which serial dilutions of the material are made in nutrient broth,[2] about 0.2 μg per ml of broth inhibited the growth of *E. coli.* It was more difficult to get a reproducible end-point in the assay of actinorubin in broth than was the case with lavendulin.

Test organism for protection studies. Klebsiella pneumoniae, American Type Culture Collection No. 8050 was used. It was found to be of serological type A by the Quellung reaction.

Technics. Dilutions of the antibiotics were prepared in sterile distilled water.

For determining toxicity a given dose of the antibiotic was injected intraperitoneally into each of 10 mice. The mice were observed daily for 14 days.

In each protection test the virulence of the

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