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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1 Kelner, A., Kocholaty, W., Junowicz-Kocholaty, R., and Morton, H. E., *J. Bact.,* 1946, **51**, 591.

2 Kelner, A., and Morton, H. E., to be published.

3 Junowicz-Kocholaty, R., and Kocholaty, W., to be published.

culture was checked. One ml amounts of an 18-hour-old broth culture of *K. pneumoniae*, serially diluted in broth, were injected intraperitoneally into mice. Usually 5 to 10 mice were injected with each dilution of the culture. At the time each dilution of the culture was injected into mice, 1 ml portions were made into poured agar plates. After 48 hours of incubation at 37°C the colonies were counted. Five to 10 bacilli, as judged by the poured agar plate count, killed over 50% of the mice, whereas 40 to 50 organisms could be depended upon to kill all of the mice. The challenging dose of culture was between 100 and 1000 times the minimum amount of culture required to kill all of the mice. For determining the therapeutic effect of the antibiotic, 100 to 1000 lethal doses of *K. pneumoniae* were injected intraperitoneally into each mouse, followed in a few seconds by an intraperitoneal injection of the drug. Usually 10 mice were injected with each graded dose of the drug. The mice were kept under observation for 14 days. The heart's blood of some of the animals dying during the course of the experiment was streaked on agar plates to determine that death was due to infection with *K. pneumoniae*.

Results. The results of injecting mice with varying dilutions of lavendulin are summarized in Table I. Intraperitoneal injection of 0.5 mg was fatal to all of 10 mice. The results of the therapeutic action of lavendulin in mice against at least 100 lethal doses of *K. pneumoniae* are summarized in Table II.

TABLE I.
Results of Injecting Mice with Varying Doses of Lavendulin.

No. of mice	Dose of lavendulin	Results
10	1 mg	1 died, < 46 hr
		1 " 53 "
		7 " < 70 "
		1 " 70 "
10	0.75 "	1 " 50 "
		6 " < 70 "
		1 " < 72 "
		2 " <104 "
10	0.5 "	6 " < 70 "
		2 " <104 "
		1 " <105 "
		1 " 125 "
10	0.25 "	1 " < 71 "
		3 " <104 "
		1 " on 6th day
		5 alive after 14 days*
10	0.1 "	10 " " 14 " †
10	75 μg	10 " " 14 " ‡
10	50 "	10 " " 14 " §

* Wt at time of injection, 17-18 g. Avg wt at end of exper., 16.9 g.
† Wt at time of injection, 17-18 g. Avg wt at end of exper., 19.3 g.
‡ Wt at time of injection, 17-18 g. Avg wt at end of exper., 20.6 g.
§ Wt at time of injection, 17-18 g. Avg wt at end of exper., 20.4 g.

TABLE II.
Summary of Tests Demonstrating the Protective Action of Lavendulin Against *K. pneumoniae* in White Mice.

Dose of culture, ml	diln	Dose of lavendulin	No. of mice injected	No. of mice dying	No. of mice surviving
1	10^{-8}	0	5	3	2
1	10^{-7}	0	5	5	0
1	10^{-6}	0	5	5	0
1	10^{-5}	0	5	5	0
1	10^{-5}	200 μg	10	0	10
1	10^{-5}	100 "	10	0	10
1	10^{-5}	50 "	10	0	10
1	10^{-5}	25 "	10	1*	9
1	10^{-5}	10 "	10	2†	8
1	10^{-5}	5 "	10	7‡	3

* Died on the 12th day of the experiment. No *K. pneumoniae* in culture from the heart's blood.
† Died on the 2nd and 3rd days of the experiment. One mouse autopsied and *K. pneumoniae* cultivated from the heart's blood.
‡ *K. pneumoniae* cultivated from the heart's blood of each of 3 mice autopsied.

TABLE III.
Results of Two Experiments in Which Mice Were Injected Intraperitoneally with Varying Doses of Actinorubin.

No. of mice	Dose of actinorubin	Results
	mg	
10	3.43	10 died (3rd and 4th days)
10	1.37	10 died (3rd, 4th, and 5th days)
10	1.0	9 died (4th, 5th, and 9th days) 1 alive 14th day
10	0.68	5 died (5th, 6th, 7th, and 10th days) 5 alive 14th day
9	0.34	9 alive 14th day
10	1	8 died on 5th day 1 died on 7th day 1 alive after 14 days*
10	0.75	3 died on 5th day 7 alive after 14 days†
10	0.5	3 died (8 to 13 days) 7 alive after 14 days‡
10	0.35	2 died, 3rd and 8th day 8 alive after 14 days§
10	0.25	All alive after 14 days§

* Wt at time of injection, 18-19 g. Wt at end of exper., 15 g.

† Wt at time of injection, 18-19 g. Avg wt of 5 mice at end of exper., 15.1 g.

‡ Wt at time of injection, 17-18 g. Avg wt at end of exper., 16.8 g.

§ Wt at time of injection, 17-18 g. Avg wt at end of exper., 19.9 g.

All of 10 mice were protected from death due to the test organism by 25 μg of lavendulin. The ratio of the therapeutic dose to toxic dose of lavendulin is of the order of 1:20. Calculating the LD_{50} of lavendulin and that dose of lavendulin which protected 50% of the mice in the presence of more than 1000 LD_{50} of culture by the procedure of Reed and Muench,[4] the ratio of therapeutic dose to toxic dose is of the order of 1:27.

The results of injecting mice with varying dilutions of actinorubin are summarized in Table III. All of 10 mice were killed by

[4] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

TABLE IV.
Summary of the Tests Demonstrating the Protective Action of Actinorubin Against *K. pneumoniae* in White Mice.

Dose of culture ml	Dose of culture diln	Dose of actinorubin	No. of mice injected	No. of mice dying	No. of mice surviving
		Date of test: April 13, 1946.			
1	10^{-9}	0	5	1	4
1	10^{-8}	0	5	5	0
1	10^{-7}	0	5	5	0
1	10^{-6}	0	5	3	2
1	10^{-5}	0	5	5	0
1	10^{-6}	340 μg	5	0	5
1	10^{-6}	137 "	5	0	5
1	10^{-6}	68.5 "	5	0	5
1	10^{-6}	34 "	5	0	5
1	10^{-6}	13.7 "	5	0	5
1	10^{-6}	6.85 "	5	1	4
1	10^{-6}	3.4 "	5	2	3
		Date of Test: April 24, 1946.			
1	10^{-9}	0	10	1	9
1	10^{-8}	0	10	7	3
0.5	10^{-7}	0	10	9	1
1	10^{-7}	0	10	10	0
1	10^{-6}	0	10	10	0
1	10^{-5}	0	10	10	0
1	10^{-5}	40 μg	10	3	7
1	10^{-5}	30 "	10	5	5
1	10^{-5}	20 "	10	6	4
1	10^{-5}	14 "	10	6	4
1	10^{-5}	10 "	10	7	3
1	10^{-5}	5 "	10	10	0
1	10^{-5}	2.5 "	10	9	1

an intraperitoneal injection of 1.37 mg. The results of one test (on April 13) on the therapeutic action of actinorubin in mice against 100 to 1000 lethal doses of *K. pneumoniae* are summarized in Table IV. All of 5 mice were protected from death by 13.7 μg of actinorubin. The ratio of the therapeutic dose to the toxic dose of actinorubin in this experiment was of the order of 1:100. No explanation is offered of the fact that 2 out of the 3 mice, which received a dose of culture estimated to be in excess of 100 lethal doses of *K. pneumoniae*, failed to die.

Repeating the experiment in part, using 100 lethal doses of *K. pneumoniae*, gave less protection than in the previous test. Comparing the dose of 30 μg of actinorubin which protected 50% of the mice against 100 lethal doses of culture with 680 μg which killed 50% of the animals gives a ratio of 1:22. Practically the same ratio was obtained using LD_{50} as calculated by the method of Reed and Muench.[4] The effects of aging and repeated freezing and thawing of the solutions of actinorubin were not determined.

Since actinorubin appeared more active *in vivo* against *K. pneumoniae* than lavendulin, 2 preliminary tests were made to determine rate of absorption following intraperitoneal or oral administration. Each of 8 mice were injected intraperitoneally with 1.3 mg of actinorubin. This would ordinarily inhibit the growth of *E. coli in* Bacto-nutrient broth, pH 7.3, in a dilution of about 1:6875. After varying intervals of time one of the mice was anesthetized with diethyl ether and bled from the axilla according to the technic described by Kuhn.[5] Fifteen minutes after the intraperitoneal injection, the blood of a mouse inhibited *E. coli* in a dilution of 1:320; after 30 minutes, the blood of another mouse inhibited in a dilution of 1:160. After 75 minutes the blood of another mouse inhibited the test organism in a dilution of 1:10. No actinorubin was detected in a 1:10 dilution of blood samples taken from mice at 2-, 3-, 4-, 5- and 6-hour intervals.

[5] Kuhn, L. R., *Science*, 1946, **93**, 504.

Seven mice were each given 3.43 mg of actinorubin in 0.25 ml by stomach tube. No actinorubin was detectable in the blood when tested after ¼, ½, 1, 2, 3, 4, and 5 hours, one animal being sacrificed at each period.

Pathological Changes.† Mice which survived the larger test injections of lavendulin and actinorubin (Tables I and III) failed to gain weight or even lost weight during the 2 weeks of observations. Postmortem examination of 6 mice 16 days after the intraperitoneal injection of each with 0.75 mg of actinorubin revealed the following abnormalities: Body weights varied from 13.4 to 19.7 g (normal weight for the same lot and age was 31 g). The livers were irregularly distorted with a reduction in the number of lobes or fusion of the lobes to form a rather solid mass which often completely enclosed the gall bladder. Microscopic examination revealed eosinophilic, necrotic foci in some livers of the animals but in all fibrous tissue stroma was irregularly increased and the lobules often were more or less distorted. A true cirrhosis was not developed, however. The kidneys were enlarged and of a pale, dull brown color. In all of the animals the convoluted tubules were dilated and lined by flattened epithelial cells, whereas the glomeruli were lined by columnar cells. The dilated tubules in 4 animals contained dense eosinophilic casts. In each of the 6 animals the thymus was reduced more or less but in 4 of the animals little remained but reticulum and epithelial elements. The other viscera were unchanged.

Five mice which survived 0.25 mg of lavendulin were sacrificed 15 days after the intraperitoneal injection of the drug. Body weights varied from 12.9 to 19.9 g (normal weight for the same lot and age was 31 g). The livers were distorted to a lesser degree than in the group which received actinorubin although the abnormalities were of the same type. The kidneys were unchanged. The thymus in 3 of the animals appeared reduced in size but microscopic sections were es-

† We are indebted to Dr. Herbert Ratcliffe, Department of Pathology, University of Pennsylvania, School of Medicine, for the examination of some of the test animals.

sentially normal. The spleens were reduced in size and the capsule was thickened by fibrous tissue growth.

These observations indicated that the liver was extensively damaged by the substances injected but regenerated more or less completely by the end of 2 weeks. Actinorubin also caused tubular damage in the kidneys and largely destroyed the lymphoid elements of the thymus. Lymphoid tissues of the spleen did not appear to have been damaged. Fibrosis of the splenic capsule may be attributed to the intraperitoneal injection of the material. Although the mice which received actinorubin showed the more extensive pathological changes, it must be borne in mind that, on a weight basis, these mice received three times more drug than the mice which received lavendulin.

Summary. The toxic doses (LD_{100}) of actinorubin and lavendulin have been determined for white mice by intraperitoneal injection. In the case of lavendulin it was 0.5 mg, while in the case of actinorubin it was 1.37 mg. The ability of the antibiotics to protect mice from death following the intraperitoneal injection of 100 to 1000 M.L.D. of *K. pneumoniae* was determined. In the case of lavendulin this therapeutic dose was 25 μg and in the case of actinorubin it was found to be 13.7 μg in one experiment. In a second experiment 0.68 mg of actinorubin killed 50% of the mice while 30 μg protected 50% of the mice against 100 lethal doses of *K. pneumoniae*. Further testing of the 2 antibiotics has not been possible due to the limited supplies of the substances.

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Production of Increased Capillary Fragility in Rats Following Irradiation.*

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In the course of a clinical study of increased capillary fragility, it became desirable to develop a corresponding study in an experimental animal. A method for measuring the increase in fragility was needed as well as a means for producing increased fragility. Hundreds of unsuccessful experiments were conducted in rats and guinea pigs before the problem was satisfactorily solved. Only the successful method, however, will be described.

Method for measuring capillary fragility in rats. Under general anesthesia the peritoneal cavity is opened by a long midline longitudinal incision, care being taken to exert no traction on the peritoneum. The abdominal wall is carefully folded back until an artery and vein are disclosed. Then, under a good light, the peritoneum is grasped on each side of the vessels and stretched between the fingers so that the lumen of the vein is obliterated but the artery can still be seen pulsating. The tension is maintained for 2 minutes after which it is relaxed. The peritoneal area supplied by the vessels is carefully inspected and, if the fragility is increased, one or more petechia will appear distal to the point of venous obstruction, usually entirely separate from any visible vessel. Actually, in a positive case, 6 or 8 such hemorrhages usually occur. If desired, the process can be repeated in the same animal, using other vessels. No petechial hemorrhages occur in the normal animal.

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