*Summary.* The repeated intravenous administration of increasing doses 0.5 to 4.0 g of *p*-aminophenol to a patient with gout did not affect the uric acid output in the urine or the blood uric acid level; occasionally there was transient elevation of temperature and nausea. Free iodine, in .008 M solution, caused an 88% inhibition of milk xanthine oxidase but was not specific for this enzyme. 0.001 M pyrogallol inhibited xanthine oxidase approximately 44% and had no effect upon other enzymes like uricase. Many other substances were tested and found to cause no appreciable inhibition of xanthine oxidase.

## 15060 P

## Acute and Subacute Toxicity of Pure Citrinin.

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Citrinin is an antibiotic produced by *Penicillium citrinum*,[1] and by *Aspergillus sp.* of the *candidus* group.[2] Robinson[3] has reported on the acute toxicity of citrinin in mice following oral and intraperitoneal administration. Timonin and Rouatt[4] have reported toxicity data obtained by Noble, who administered citrinin intraperitoneally and subcutaneously to mice, intraperitoneally to rats, and orally and subcutaneously to rabbits. In the following summary the data of these investigations have been recalculated in terms of milligrams per kilogram of body weight so as to make them directly comparable with the data presented in this report.

Because of the low solubility of citrinin in water, Robinson[3] administered it to mice as a suspension in 10% gum acacia solution. In a series of 30 mice he gave the suspended citrinin orally in doses ranging from 12.5 to 500 mg per kilo of body weight. Doses of 100 mg or less produced no fatalities, but 200 and 500 mg killed 40 and 100% of the mice, respectively, in 7 days. In another series of 30 mice he found 20% and 100% mortality resulting from intraperitoneal administration of 200 and 400 mg, respectively.

In the toxicity tests reported by Timonin and Rouatt[4] the citrinin was dissolved in sodium hydroxide (pH 8-9.5) and the solution adjusted to a neutral reaction as rapidly as possible with hydrochloric acid. A series of 20 mice were given citrinin intraperitoneally in doses ranging from 25 to 125 mg per kilo of body weight. All mice receiving 100 mg died in 6 to 18 hours, and all mice receiving 125 mg died in 5 to 6 hours. None of the mice given 50 mg died after a single administration, but repetition of this dose 24 hours later produced 40% mortality. In a series of mice receiving 100 mg subcutaneously, 80% mortality resulted in 4 to 24 hours. No deaths occurred in 5 rats given 50 mg per kilo intraperitoneally. One rabbit receiving 25.7 mg per kilo in 2 doses by mouth and a second rabbit given 30 mg (absolute dose) subcutaneously daily for 5 days showed no ill effects.

Unless adequate precautions are taken, citrinin is apt to be contaminated with oxidation products and other impurities derived from the culture medium. Even pure citrinin is contaminated readily with oxidation products when it is dissolved in alcohol or in aqueous solution on the alkaline side of neutrality. The high degree of purity of the citrinin used in obtaining the following toxic-

[1] Hetherington, A. C., and Raistrick, H., *Philosophical Transactions*, 1931, **220**, 269.

[2] Timonin, M. I., and Rouatt, J. W., *Canadian J. Public Health*, 1944, **35**, 80.

[3] Robinson, H. J., A Thesis submitted to the Graduate Faculty of Rutgers University, New Brunswick, N.J., May, 1943.

[4] Timonin, M. I., and Rouatt, J. W., *Canadian J. Public Health*, 1944, **35**, 396.

TABLE I.
Composition and Physical Characteristics of Pure Citrinin.

| Product | % carbon | % hydrogen | Melting point |
|---|---|---|---|
| Theoretical | 62.37 | 5.64 | — |
| Hetherington and Raistrick[1] | 62.20 | 5.63 | — |
| " " " | 62.32 | 5.58 | 166-170 |
| Prepared by sublimation | 62.40 | 5.72 | 167-170 |
| Prepared by extraction | 62.20 | 5.66 | 167-170 |

TABLE II.
Toxicity of Citrinin for Rats, Guinea Pigs, and Rabbits.

| Mode of administration | Dose, mg/kg | No. of animals | No. of dead in days 1 | 7 | 14 |
|---|---|---|---|---|---|
| Rats | | | | | |
| Subcutaneous | 25 | 8 | 0 | 0 | 0 |
| | 50 | 15 | 0 | 3 | 3 |
| | 60 | 8 | 0 | 3 | 3 |
| | 75 | 8 | 3 | 5 | 5 |
| | 100 | 9 | 9 | | |
| | 200 | 5 | 5 | | |
| Intraperitoneal | 25 | 8 | 0 | 0 | 0 |
| | 50 | 8 | 0 | 0 | 0 |
| | 60 | 9 | 1 | 2 | 2 |
| | 75 | 12 | 11 | 12 | |
| | 100 | 4 | 4 | | |
| Guinea pigs | | | | | |
| Subcutaneous | 25 | 5 | 0 | 0 | 0 |
| | 50 | 5 | 0 | 5 | |
| | 75 | 4 | 1 | 3 | 3 |
| | 100 | 3 | 1 | 3 | |
| Rabbits | | | | | |
| Intravenous | 5 | 5 | 0 | 0 | 0 |
| | 10 | 5 | 0 | 0 | 0 |
| | 15 | 5 | 0 | 1 | 1 |
| | 20 | 5 | 0 | 3 | 3 |
| | 25 | 1 | 0 | 1 | |
| | 50 | 5 | 0 | 0 | 5 |
| | 100 | 4 | 4 | | |
| | 150 | 1 | 1 | | |

ity data is shown in Table I. The citrinin was produced by *Penicillium citrinum* grown on asparagus juice medium by Humfeld and Feustel.[5] Citrinin purified by sublimation* under reduced pressure and by extraction has a bright lemon color with no suggestion of a brown or orange tint. The purification by extraction will be described by us in a later publication.

An aqueous solution of citrinin for toxicity studies was prepared immediately before use by dissolving a weighed amount of citrinin in the theoretically correct volume of standardized normal sodium hydroxide. With the aid of a glass electrode the pH of the solution of the sodium salt was adjusted to neutrality. Any unused solution was acidified and the citrinin recovered.

The acute toxicity of citrinin was determined in 94 rats, 17 guinea pigs, and 31 rabbits. The citrinin was administered subcutaneously to 53 rats and intraperitoneally to 41 rats. The subcutaneous route was used in guinea pigs, and the intravenous route in rabbits. The data obtained are summarized in Table II.

The subacute toxicity of citrinin injected

[5] Humfeld, H., and Feustel, I. C., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 232.

* Purification of citrinin by sublimation was carried out by Dr. F. T. Jones of the Western Regional Research Laboratory. Details of the physical properties and methods of purification of citrinin are being prepared for publication.

intravenously was studied in 6 rabbits. Daily injections, except for Sunday, were continued for 8 weeks. Three rabbits received 5 mg per kilo daily for 26 days. For the next 13 days the dosage was raised to 15 mg, and finally to 20 mg for the next 13 days. Three other rabbits were given 10 mg daily for 26 days, and 20 mg daily for the next 26 days. There were no fatalities in these experiments on subacute toxicity.

The symptoms observed in these toxicity studies will be discussed in a later publication dealing with the tissue changes produced by citrinin, and with the pharmacological data covering effects on blood, circulation, respiration, and smooth muscle structure.

*Conclusions.* The lower toxicity values reported by Robinson, as compared with those reported by Timonin and Rouatt and those reported here, may be due to a low rate of absorption of citrinin from a suspension in gum acacia solution.

Citrinin in solution is rapidly absorbed, regardless of the mode of administration, as shown by the toxicity data.

The production of tissue changes and the fact that citrinin may result in delayed deaths, up to fourteen days, would make a statement regarding an $LD_{50}$ dose misleading.

## 15061

## Effect of Streptomycin on Avian Malaria.

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Streptomycin, an antibiotic produced by the soil actinomycete *Streptomyces griseus,* was first described by Schatz, Bugie, and Waksman.[1] Unlike penicillin, streptomycin has been shown to have a high order of activity against certain gram negative infections both *in vitro* and *in vivo*.[2,3,4] Streptomycin has also been found to be active against the acid fast organism *Mycobacterium tuberculosis* both *in vitro*[5] and in the experimental animal.[6] In this communication we are reporting the results of studies on the activity of this antibiotic against avian malaria.

Trophozoite induced *Plasmodium gallinaceum* infections were established in 50 g Single Comb White Leghorn chicks by an intravenous inoculum of 200,000,000 parasitized erythrocytes per kilogram. The tests for suppressive activity against both trophozoite induced *P. cathemerium* and *P. lophurae* malaria were performed on 50 g Pekin ducklings inoculated intravenously with 500,000,000 parasitized erythrocytes per kilogram. The sporozoite induced *P. gallinaceum* infections used for the prophylactic tests were established in 50 g Single Comb White Leghorn chicks by the intravenous inoculation of 0.2 cc per bird of a suspension of sporozoites prepared by grinding 100 infected mosquitoes in 20 cc of chicken plasma.

The streptomycin used in these experiments was supplied by Merck & Co., Inc. and came from batch 162 which had a potency of 440 units per mg.[2] In all cases the streptomycin was administered as an aqueous solution intramuscularly every 3 hours. Birds inoculated with the trophozoites of *P. cathemerium, P. gallinaceum* and *P. lophurae* were treated for 5 days while the birds inoculated with the

[1] Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

[2] Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A., *Science*, 1944, **100**, 103.

[3] Robinson, H. J., Smith, D., and Graessle, O., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 226.

[4] Heilman, F. R., *Proc. Staff Meet., Mayo Clinic*, 1944, **19**, 553.

[5] Schatz, A., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 244.

[6] Feldman, W. H., and Hinshaw, H. C., *Proc. Staff Meet., Mayo Clinic*, 1944, **19**, 593.