the findings described above. The pentobarbital control showed slight cloudy swelling of the kidney with casts in many of the loops of Henle. Other organs were normal or showed varying degrees of passive congestion.

Discussion. Surgical methods produce a uremia which is complicated by the dangerous and uncertain post-surgical state. Uremia produced chemically is often complicated by toxic reactions and uncertainty of results. The use of sodium tetrathionate as a nephrotoxin appears to overcome these disadvantages. The cause of the specific susceptibility of the tubule cells to the action of tetrathionate is not yet known. Gilman *et al.*(1) have discussed the possible mode of action of tetrathionate. The fact that the specific lesions produced by tetrathionate are limited to the kidney allows the production of experimental uremia without complications resulting from the technic used. The development of chronic uremia in one dog may have resulted from the contraction of an upper respiratory infection at a time when renal tubular proteins were circulating in the blood stream, with resultant formation of autoantigens and the subsequent development of auto-antibodies specific for the renal tubule.

Although one laboratory procedure, such as an NPN determination, cannot indicate the complete functional status of an organ as complex as the kidney, it can, when performed daily indicate the renal trend. Fig. 1 shows that increasing the dose of sodium tetrathionate from 100-500 mg/kilo of body weight increases the duration and severity of the uremia as indicated by the NPN and incidence of death. Those succumbing to lower doses were older animals; those surviving higher doses were much younger. This is in accordance with the findings of MacNider (5) who demonstrated that older dogs are more susceptible to nephrotoxins than younger ones.

The appearance of urinary reducing substances following tetrathionate administration required the ruling out of a hyperglycemia. In the control animal, fasting blood sugars were at low normal levels when fresh urines gave a 3 or 4 plus reaction with Benedict's solution, indicating that the reducing substances in the urine were due to tubular lesions and not a hyperglycemia.

Summary and conclusions. Uremia was produced in dogs by intravenous administration of varying doses of sodium tetrathionate. The findings of Gilman *et al.*(1) cited above were confirmed. In addition, it was shown that renal lesions resulting from tetrathionate administration, heretofore not described in detail, involve the distal convoluted and collecting, as well as the previously mentioned (1) proximal renal tubules. The resulting uremia may be rapidly fatal or continue 20 or more days, and is accompanied by non-hyperglycemic urinary reducing substances and lowered CO_2CP and SpG of the urine. The mechanism of chronicity following uremia complicated by an upper respiratory infection is briefly discussed. The specific action of tetrathionate on the kidney and the freedom from non-uremic complications following its use demonstrates its efficacy for producing experimental uremia.

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Reversible Inhibition of Growth of *Microsporum audouini* with Neopyrithiamine.* (18486)

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Recent studies of the nutritional requirements of certain fungi have definitely established the essential nature of certain metabolites for growth and formation of spores. It has been shown that certain fungi are unable to synthesize these essential metabolites

TABLE 1. Activity of Neopyrithiamine and Neopyrithiamine + Thiamine on Growth of *Microsporum audouini* and *Microsporum canis* from Infected Hairs.

μg per ml		*Microsporum audouini* No. 1	*Microsporum audouini* No. 2	*Microsporum canis*
Neopyrithiamine	Thiamine			
Control		++++	++++	++++
5		+ m	0	++
3		+ m	+ m	++
1		+ m	+ m	++
0.5		+ m	+ m	++++
3	.3	++++	++++	++++
3	.1	++++	++++	++++
3	.03	++++	++++	++++
3	.01	++	++++	++++

m = microscopic.

(amino acids, vitamins and so forth) and these deficient organisms will not grow unless the medium contains all factors essential for growth. Moreover, even in the presence of the essential factors, growth is prevented when the medium also contains compounds known as metabolic antagonists, which specifically interfere with the utilization of these factors by the organism(1). Unfortunately, little information is available regarding the factors essential for growth of pathogenic fungi attacking human beings. Conant(2) in his study of the microspora noted that *Microsporum canis* grew profusely on polished rice grains but that *Microsporum audouini* grew poorly and did not produce aerial growth or macroconidia. Further observations by Benedek(3), Hazen(4), de Arêa Leão and Cury(5) and Walker(6) suggested that *Microsporum audouini* was a deficient organism.

The following report demonstrates the inhibition of growth of *Microsporum audouini* with neopyrithiamine, a metabolic antagonist of thiamine. Thiamine, therefore, appears to be essential for growth of this organism.

Method. One stock strain of *Microsporum audouini* and the spores on fluorescent hairs from 3 patients, subsequently proved to be 2 strains of *Microsporum audouini* and 1 strain of *Microsporum canis,* were tested for their ability to grow on Sabouraud's dextrose agar (pH 6.8) which contained various concentrations of neopyrithiamine or of neopyrithiamine and thiamine. The stock medium used for testing was 20% more concentrated than the standard stock medium so that after addition of the reagent solutions, standard concentrations of nutrients would result. Serial dilutions of the reagents were prepared by placing the proper aliquot parts of reagents in Petri dishes and mixing them with 8 ml of the medium. The volume was kept constant at 10 ml by the addition of the proper amount of sterile distilled water. The stock strain of *Microsporum audouini* was heavily inoculated onto the plates as 0.2 ml of a ground suspension prepared from a week-old culture growing on Sabouraud's dextrose agar. The two strains of *Microsporum audouini* and one strain of *Microsporum canis* on fluorescent hairs were tested by placing several infected hairs on duplicate plates. The hairs were examined microscopically after they had been placed on the agar to determine whether or not spores were present. The plates were incubated at room temperature and examined periodically for growth for one month. Hairs were transplanted to Sabouraud's dextrose agar from one plate of the duplicate sets which did not show macroscopic growth in 14 days

* We are indebted to Dr. H. J. Robinson of Merck and Co., Inc., for the neopyrithiamine used in this study.

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3. Benedek, T., *Mycologia,* 1943, v35, 222.

4. Hazen, Elizabeth L., *Mycologia,* 1947, v39, 200.

5. de Arêa Leão, A. E., and Cury, Amadeu, *Mycopathologia and Mycologia Applicata,* 1950, v5, 65.

6. Walker, Jacqueline, *Brit. J. Dermat. and Syph.,* 1950, v62, 395.

TABLE II. Activity of Neopyrithiamine and Neopyrithiamine + Thiamine on Growth of a Stock Strain of *Microsporum audouini* on Solid and Liquid Media.

μg per ml		Growth	
Neopyrithiamine	Thiamine	Liquid medium	Solid medium
Control		++++	++++
.8		±	0
.4		±	+ m
.2		±	++
.8	.8	++++	++++
.8	.08	++++	++++
.8	.008	++++	++++

m = microscopic.

(Table I). The stock strain also was tested in liquid Sabouraud's dextrose medium. Dilutions of the test reagents were prepared in the same manner as for the Petri plates but in 28 by 100 mm tubes with a screw cap, which remained loose to allow aeration of the tube.

Results. The stock strain on solid medium did not show growth which could be detected either macroscopically or microscopically at a concentration of 0.8 μg per ml of neopyrithiamine (Table II). Growth was visible only microscopically at a concentration of 0.4 μg per ml of neopyrithiamine, but, although filaments began to develop, the amount of growth never became visible macroscopically even after a month of incubation. Growth which could be seen easily macroscopically was observed at a concentration of 0.2 μg per ml of neopyrithiamine but the amount of growth did not equal that on the control plate or on plates to which thiamine was added. This inhibition of growth by neopyrithiamine could be reversed by the addition of thiamine to the medium. Addition of as little as 0.008 μg per ml of thiamine could overcome the effects of 0.8 μg per ml of neopyrithiamine.

Neopyrithiamine was not as effective as an inhibitor in liquid medium as on the agar plates. At 0.8 μg per ml of neopyrithiamine, visible growth was evident after 4 days' incubation but growth did not continue as in the control tubes. After one month's incubation the medium in the control tubes was completely filled with mycelium and was covered over by a heavy pellicle. Tubes containing neopyrithiamine had no more growth after one month of incubation than was observed after 4 days. Neither the rate nor the amount of growth differed between control tubes and those containing 0.8 μg of neopyrithiamine and 0.8 μg per ml of added thiamine.

Results of planting hairs infected with *Microsporum audouini* or *Microsporum canis* on Sabouraud's dextrose agar containing various concentrations of neopyrithiamine or neopyrithiamine plus thiamine are recorded in Table II. It appears that higher concentrations of neopyrithiamine are required to inhibit the growth of *Microsporum audouini* from infected hairs than in pure culture transplants. Strain No. 1 of *Microsporum audouini* is less sensitive than strain No. 2 to the inhibitory effect of neopyrithiamine but also requires smaller amounts of thiamine to overcome the inhibition. Growth of *Microsporum canis* is somewhat retarded at a concentration of 1 μg per ml of neopyrithiamine but macroscopic colonies are easily visible in ten days and growth continues with incubation of the plates. Except for strain No. 2 originally planted on medium containing 5 μg per ml of neopyrithiamine, all hairs transplanted to Sabouraud's dextrose agar grew out in 4 days. After one week of incubation the hairs were removed from the one transplant which did not grow and again examined microscopically. The shafts were covered with the typical spore mosaic but germination was not evident.

Comment. Several vitamins have been reported as essential for the growth of *Microsporum audouini*(5). The inability of Hazen (4) to increase growth by the addition of thiamine and pyridoxine to a medium of honey agar does not eliminate these vitamins from the essential list. The quantity of factors producing growth which were present in the honey was not known. The results of Walker(6) show the requirement of these factors for some strains. The ability of neopyrithiamine to inhibit the growth of pure cultures of *Microsporum audouini* indicates a requirement for thiamine. This organism either cannot synthesize the vitamin or it produces thiamine so slowly that growth is hindered. The ability of the organism to in-

itiate but not to continue growth may be owing to the preformed thiamine contained within the organism at the time of transfer. Growth ceases or becomes extremely slow after this supply is exhausted. The growth of *Microsporum audouini* from infected hairs in concentrations of neopyrithiamine higher than that which completely inhibits pure culture transplants may be brought about by higher concentrations of thiamine present on the hair.

Unfortunately Sabouraud's dextrose agar is not devoid of thiamine. Stokes and coworkers(7) have found 0.11 to 0.28 μg of thiamine per gram of peptone. Assuming that the peptone used in this study contained the lower concentration of thiamine, each milliliter of medium would already contain 0.001 μg of thiamine. It seems reasonable to assume that on a purely synthetic medium entirely lacking in thiamine, still lower concentrations of neopyrithiamine would inhibit growth. An attempt has not been made to calculate the inhibition index from these data as the exact concentration of thiamine was not known. Experiments are being carried out on a synthetic medium to determine the index. It appears, with one exception, that the concentrations of neopyrithiamine employed did not kill all the *Microsporum audouini* during 14 days' contact. Strain No. 2 originally planted on medium containing 5 μg per ml indicates, however, that in higher concentrations neopyrithiamine may be fungicidal in the same period.

Summary. The ability of neopyrithiamine to inhibit growth of *Microsporum audouini in vitro* in very low concentrations suggests its use in cases of tinea capitis caused by this fungus. The application of neopyrithiamine alone or in combination with other agents or vitamin antagonists now being studied may prove helpful in combating these infections.

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Vitamin B_{12}, a Factor in Prevention of Hydrocephalus in Infant Rats.* (18487)

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During a study of the adequacy of a synthetic-type, casein diet for reproduction and lactation in the rat, Richardson and Hogan (1) observed that about 2% of the offspring were hydrocephalic. They ascribed this abnormality to a nutritional deficiency and showed that it could be prevented by an aqueous extract of liver. O'Dell, Whitley and Hogan(2) showed that the addition of folic acid to a diet containing casein as the source of protein usually, but not always, prevented the abnormality. More recently Hogan, O'Dell and Whitley(3) observed hydrocephalus in about 20 percent of the offspring when a folic acid inhibitor, crude methylpteroylglutamic acid (Methyl PGA), was added to a diet that contained soybean oil meal as the source of protein. The type of diet consumed during the pre-experimental period had a marked effect on the incidence of hydrocephalus among the first few litters produced by each dam, a fact which suggested that a protective factor may be stored in the

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