Wellcome and Co. through the courtesy of Edwin J. de Beer. Dextran was provided by Abbott Laboratories; molecular weight 79, 400, low fraction, 28, 300, high fraction 180, 800. 5-Hydroxytryptamine was serotonin creatine sulfate (Nutritional Biochemical Co.). This work was partially aided by grants from the Ministry of Health of the Province of Quebec (Federal-Provincial Health Research Grants).

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Protection against X-irradiation by 3-Amino-1,2,4-triazole

Heim, Appleman, and Pyfrom (*1*) recently demonstrated that after intraperitoneal injection of 3-amino-1,2,4-triazole (AT) into rats, the liver and kidney catalase activity was sharply reduced. Our first conclusion, of course, was tentatively to consider this compound to be a catalase inhibitor. Since such radiation protectors as azide (*2*) and cyanide (*2, 3*) have also been shown to inhibit catalase *in vitro* (*4*) and, at least in the case of azide, *in vivo* (*5*), it was decided to test AT also as a protective agent against ionizing radiation (*6*). Injection of a catalase inhibitor may appear to be paradoxical in attempting to protect against irradiation lethality. However, one must consider such possible mechanisms as the formation of a catalase-inhibitor complex more radiation-stable than the uncomplexed catalase (*7*).

Table 1 indicates that intraperitoneal injection of AT before whole body x-irradiation rather consistently protects a large percentage of mice against 650 r of x-rays and significantly prolongs the survival of animals that receive 750 or 850 r of x-rays. (If all data shown are pooled for mice receiving 850 r alone, and for mice receiving AT followed by an 850-r dose, the Student's t value for the difference in survival time is 2.90, with a total population in each case of 59 mice killed. This represents $0.001 < P < .005$.) If administered before a 1700-r dose, or after any dose of x-rays, AT is without effect. Even if AT is administered as long as 24 hours before the irradiation, some prolongation of survival time is conferred. It might be mentioned that the doses of AT employed were well tolerated by the mice.

Even though AT per se has been found not to be an inhibitor of catalase (*8*), the possibility cannot be excluded that a catalase mechanism is in some way relevant to the radiation protection, for a single injection of this compound will cause a 65-percent reduction in liver catalase activity as late as 24 hours after injection (*8*). The mechanism of the biological effects of AT is presently being further investigated.

After this work had been completed, a paper appeared by Friedberg (*9*), indicating no significant effect of AT on mortality rate after 934 r. His data do show, as do ours, prolongation of survival time.

Table 1. Protection of mice by 3-amino-1,2,4-triazole (AT) against whole body x-irradiation.

Amino-triazole (mg/kg)	X-ray (r)	Order of treatment	Number of mice	30-day survivors (%)	Average survival time (days)*
		Experiment 1			
0	450		10	90	9.0
2000	450	AT, x-ray	12	92	13.0
0	650		12	0	12.5
2000	650	AT, x-ray	10	70	15.7
0	850		12	0	8.9
2000	850	AT, x-ray	12	8	12.2
4000	0		11	100	
		Experiment 2			
0	850		8	0	6.3
2250	850	AT, x-ray	12	8	9.7
2250	0		12	100	
		Experiment 3			
0	850		11	0	11.5
1817	850	AT, x-ray	11	0	13.5
1817	850	x-ray, AT	11	0	11.0
0	1700		11	0	4.5
1817	1700	AT, x-ray	11	0	4.4
		Experiment 4			
0	850		12	0	13.4
1817	850	AT, x-ray	12	17	13.3
1817	850	x-ray, AT	12	0	12.1
0	1700		11	0	4.0
1817	1700	AT, x-ray	12	0	4.5
		Experiment 5			
0	650		16	19	13.9
2000	650	AT, x-ray	16	75	19.3
0	750		16	0	11.1
2000	750	AT, x-ray	13	0	13.6
0	850		16	0	10.6
2000	850	AT, x-ray	16	0	12.0
2000	0		16	100	

* Average survival time refers only to those animals that succumbed within the 30-day period.

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11 February 1957

Use of Δ4-Cholestenone to Reduce the Level of Serum Cholesterol in Man

In 1953 Tomkins *et al.* (*1*) demonstrated that a single feeding of 4-cholestene-3-one (cholestenone) to rats reduced the capacity of their livers to convert acetate to cholesterol. Soon after this observation was made, studies were initiated in our laboratory to test the effects of prolonged administration of cholestenone on the level of plasma cholesterol.

Dogs were fed 1 g of cholestenone every 8 hours for 17 days. In one dog, the concentration of plasma cholesterol fell from an initial value of 100 mg/100 ml to 70 mg on the eighth day of feeding, to 65 mg on the 12th day, and to 55 mg on the 17th day. In another dog, the levels of plasma cholesterol were 115 before the cholestenone feeding was begun and 75, 80, and 70, respectively, on the 8th, 12th, and 17th days of feeding.

Substantial reductions in the levels of plasma cholesterol were also observed in chickens that were fed Purina broiler

chow to which had been added 1 percent cholestenone. In one bird, a 10-day feeding of this diet reduced the level of plasma cholesterol from 104 to 57 mg, and in another, a 16-day feeding period resulted in a fall in the cholesterol concentration of plasma from 131 to 58 mg/100 ml. Steinberg and Frederickson (*2*) have also shown that the feeding of 1 percent cholestenone to rats suppresses the incorporation of acetate-C^{14} into cholesterol by hepatic tissue and brings about a reduction in the levels of serum cholesterol.

Since the administration of cholestenone offered a means for reducing plasma cholesterol—which is regarded today as one of the parameters in the development of arteriosclerosis—we have carried out extensive studies on the fate of cholestenone in the body. In the rat it was shown, with the aid of cholestenone-4-C^{14}, that this steroid is converted to cholestan-3β-ol (dihydrocholesterol) (*3*). More recently, we have observed that this conversion takes place by the time the C^{14} of the ingested cholestenone-4-C^{14} appears in thoracic duct lymph (*4*). An important observation made in connection with the prolonged feeding of cholestenone in birds is that the level of *total sterols*—in contrast to cholesterol level—*is not reduced in plasma* and that large amounts of cholestanol accumulate in plasma and other tissues (*5*). The prolonged feeding of cholestanol has been shown to induce arteriosclerosis in rabbits (*6*) and chickens (*7*). Because high levels of tissue cholestanol result from the feeding of cholestenone, one would therefore expect to find that the latter is also atherogenic when it is fed in large amounts.

Procedures that lower plasma cholesterol levels are eagerly being put to use in man today. In view of this, it is necessary to call attention to the dangers that may result from prolonged administration of large amounts of a steroid like cholestenone which is converted, in the animal body, to an arteriosclerosis-inducing sterol. Our studies (*8*) also bring out that it is important to know the level of total steroids in plasma, as well as that of cholesterol, when one is considering the feeding of large amounts of steroids to influence the course of arteriosclerosis in animals.

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Uricolytic Activity of Purified Uricase in Two Human Beings

Recently, several methods for obtaining highly purified uricase have become available (*1, 2*). It was demonstrated (*3*), as had been shown with less pure enzyme (*4*), that parenteral administration of uricase could temporarily decrease serum uric acid levels in chickens. Unlike the earlier attempts, this time with much purer material, it was demonstrated in two laboratories that doses several times greater than those given the chickens, on a body-weight basis, had no anaphylactoid or other toxic properties when tested on small animals.

It was deemed reasonably safe to cautiously inoculate human beings with our purest enzymic preparation (*2*). To insure against bacterial infection from this preparation, the aqueous enzyme suspension was shaken for a few seconds with toluene. Samples were then inoculated into three guinea pigs and cultured aerobically and anaerobically. After 48 hours, when there was no evidence of bacterial growth and the animals appeared to be normal, the preparation was used. Toluene separated from the aqueous suspensions, and with care the enzyme was inoculated with very little hydrocarbon present. Relatively greater quantities of toluene in saline suspension were not toxic to mice (*3, 5*).

The first patient was a 55-year-old male (57 kg) with a long history of typical gouty arthritis, but who did not have an attack either just before or during the experimental period. He was put to bed and kept on a low purine diet during the course of the experiment. All urine was collected and analyzed for allantoin according to the method of Young and Conway (*6*). Serum uric acid levels were determined at various intervals according to Brown's method (*7*). A second patient, a 63-year-old male (71 kg) with no medical history of gout was treated the same way.

The uricase preparation was administered after the patients had been resting and had been on the low purine diet for more than 48 hours. The enzyme was administered intravenously in small doses, and each successive dose was held back until it was observed that the preceding one was producing no unexpected reactions. The preparation administered contained 104 units (*3*) and 13.5 μg of protein nitrogen per milliliter in suspension. (A unit of activity is the amount of enzyme required to break down 1 μg of uric acid per minute at 37°C and *p*H 9.2 in a solution where the initial concentration of uric acid is 5 μg/ml.

Figures 1 and 2 show the serum uric acid levels for each patient and the corresponding urinary allantoin excretions for the entire experimental period. The changes in allantoin output clearly demonstrate that following intravenous uricase injections man can convert uric acid to allantoin.

Normally, man excretes only that small quantity of allantoin which is ingested with his food. The *in vivo* uric acid breakdown was not as clearly demonstrated in either case. In each of these early experiments, it was not intended to administer a therapeutic dose of the enzyme, but simply to administer a quantity just sufficient to elicit a definite uricolytic effect. This was accomplished in both cases.

As was pointed out earlier (*3*), neither the lowering of serum uric acid level nor the measurement of allantoin formed

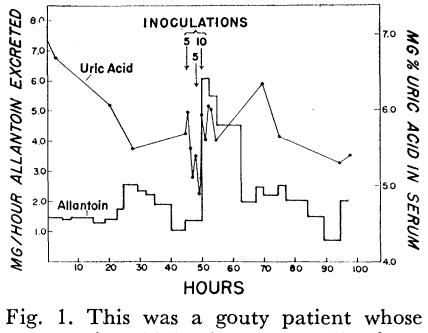

Fig. 1. This was a gouty patient whose serum uric acid levels were usually above 7.0 mg percent. The sharp decrease of uric acid level after the patient went on a low purine diet and the rapid rebound of the level after the initial drop following administration of uricase are striking. The numbers under "inoculations" represent milliliters of enzyme given.

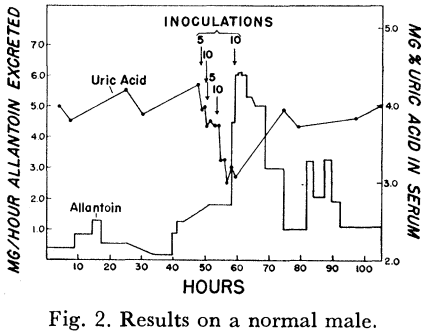

Fig. 2. Results on a normal male.