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

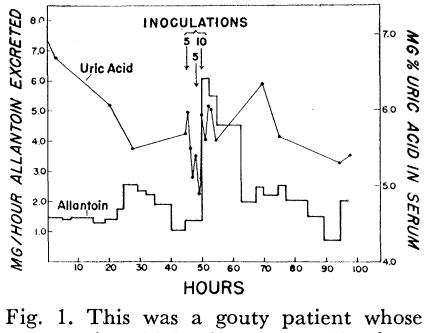

Fig. 1. This was a gouty patient whose serum uric acid levels were usually above 7.0 mg percent. The sharp decrease of uric acid level after the patient went on a low purine diet and the rapid rebound of the level after the initial drop following administration of uricase are striking. The numbers under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

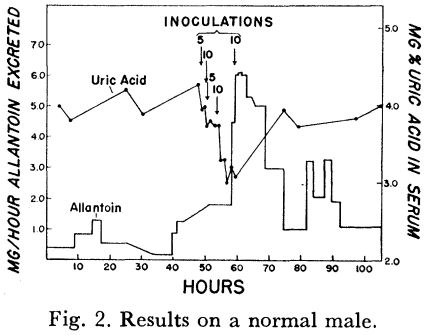

Fig. 2. Results on a normal male.

necessarily measures the uricolytic effect. In men, especially those who suffer from gout, there may be uric acid deposits. This reserve and normal endogenous and exogenous nucleic acid breakdown tend to maintain a constant serum uric acid level. Also, the breakdown of uric acid in blood does not necessarily lead to the quantitative formation of allantoin.

From the knowledge that even purer uricase preparations than that used here may be available (*1*) and that we are still far from using toxic doses, as demonstrated with animals, we are led to hope that the enzyme may yet be an adjunct in the treatment of gout and other pathological processes in which it may be necessary to clear the bloodstream of high uric acid concentration. In the rapidly developing fields of chemotherapy and radiotherapy, there is a potential need for a means of removal of excess uric acid which accumulates from the nuclear breakdown of malignant cells.

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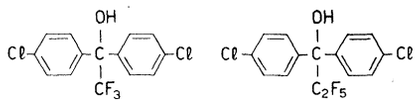
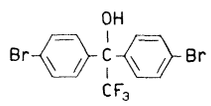
10 January 1957

Prevention of Oviposition in the Housefly through Tarsal Contact Agents

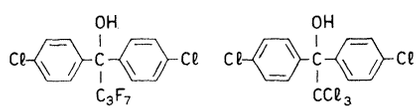
In our attempt to find ways of fighting insects, alternative to the use of contact insecticides, we have sought contact agents that would, even in sublethal doses, reduce significantly oviposition in houseflies. Among the substances tested, di-(*p*-chlorophenyl)-trifluoromethylcarbinol (I) and di-(*p*-chlorophenyl)-pentafluoroethylcarbinol (II) were outstanding. The corresponding dibromo compound (III), as well as di-(*p*-chlorophenyl)-heptafluoropropylcarbinol (IV), showed lower activity. If the chlorine atoms in I are replaced by methyl or methoxyl groups, the activity disappears completely, while replacement of the fluorine atoms in I by chlorine, which yields di-(*p*-chlorophenyl)-trichloromethylcarbinol (V) (DTMC), results in low activity. Di-(*p*-chlorophenyl)-dichloromethylcarbinol and DMC were completely inactive.



I ++++ II ++++



III ++



IV + V +

All of these compounds, which have become available only recently (*1, 2*) and have also shown interesting biological properties in other respects (*2, 3*), reduce, delay, or prevent oviposition in houseflies upon tarsal contact, when they are applied to females prior to feeding with milk.

Since compounds I (*2*) and II possess some slight contact toxicity, their oviposition-inhibiting properties are best demonstrated on a highly polyvalent resistant strain of houseflies. An extremely resistant Swiss strain (K_1) of *Musca domestica* L. (*4*) whose females are not at all affected by compounds I and II even at continuous exposure, has proved very valuable in these experiments, which have also employed a normal Swiss strain of *Musca domestica* L. and a normal and highly DDT-resistant local strain of *Musca vicina* Macq.

In general, 3-day-old females were taken from cages with mixed populations (thus fertilization was ensured) fed water and sugar only (*5*). They were then treated with compound I or II, introduced with equal numbers of untreated males into new cages, and fed with milk. Results with compounds I and II are summarized in Table 1; controls laid eggs normally.

It is thus possible to counteract the influence of continuous feeding with milk by continuous exposure to compound I or II. Smaller quantities or shorter exposure (with method 4) than those shown in Table 1 delay or drastically reduce the laying of eggs, while continuous feeding of milk, under the conditions of experiments 3 and 4 (Table 1), overcompensates the effect of compounds I and II.

On dissection of females that had been continuously exposed to compound I or II (method 5), it was found that motile spermatozoa were abundant in the spermathecae and that ovaries developed normally (same length as in control flies on milk) and contained eggs. We have thus a case of "forced retention" (*6*).

Table 1. Effect on oviposition of treatment of houseflies with compounds I and II. Three-day-old females of a highly resistant strain (K_1) of *Musca domestica* L. that had been fed only water and sugar were used. Milk was offered either daily (beginning on the fourth day of life) or only on the fourth day of life.

Expt. No. and mode of application of compounds	Milk offered	Oviposition during entire lifetime	
		Compound I	Compound II
1. Feeding in milk (0.01%)	Daily (treated)	Normal	Normal
2. Exposure to vapor	4th day	Normal	Normal
3. Topical*	4th day	None	None
4. Tarsal contact for 30 min†	4th day	None	Negligible
5. Continuous exposure‡	Daily	Very low	None

* One microgram in acetone per female.
† Females were exposed for 30 minutes to a deposit of 1 g/m² in petri dishes and then placed in the cages with the males.
‡ Filter paper of area equal to the area of one side of the cage was impregnated with 1.5 g/m² and hung in the center of the cage. (Crowding of flies in cages, which results in the covering of the compounds on the filter paper by feces, should be avoided in these experiments.)

The only data on the effect of chlorinated hydrocarbons on oviposition that have come to our knowledge are the following: Dieldrin in sublethal doses increases the reproductive potential in houseflies and *Drosophila melanogaster* Meig. (*7*), while DDT has a similar effect in *Metatetranychus ulmi* Koch (*8*). In *Drosophila* (*9*), DDT is reported to slightly reduce oviposition (*10*).

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