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Effects of Orally Administered Allopurinol (Hyroxyprazolo 3,4-d pyrimidine) on Uric Acid, Selected Blood Lipid and Hemogram Parameters in Rabbits

Gary A. Thibodeau

EFFECTS OF ORALLY ADMINISTERED ALLOPURINOL (HYDROXYPRAZOLO 3,4-d PYRIMIDINE) ON URIC ACID, SELECTED BLOOD LIPID AND HEMOGRAM PARAMETERS IN RABBITS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Head, Pharmacology Department

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INTRODUCTION

Gout is one of the earliest disease entities to be described in medical writings. Hippocrates noted familial distribution patterns and described its classic symptomatology in the 5th century B.C. Empirical treatment employing colchicine was advocated by Alexander of Tralles in the 6th century A.D. A better understanding of the complex association between clinical manifestations of gout and uric acid, carbohydrate and lipid metabolism has made rational treatment possible only very recently.

Ample experimental and clinical data now available show a definite association between disorders of purine metabolism resulting in hyperuricemia and frequent concomitant hyperlipidemia. A high incidence of both hypercholesterolemia and hypertriglyceridemia has been documented.

Elevated serum lipids may explain, in part, clinical manifestations of atherosclerosis, occlusive peripheral and coronary arterial disease and vascular nephrosclerosis so common in approximately 800,000 individuals suffering from gout in the United States. Renal failure, ischemic and degenerative cardiovascular disease are the most common causes of death in these patients. Therefore, the need for safe and reliable drugs that may be employed in the rational treatment of both gout and associated cardiovascular alterations is abundantly clear.

Allopurinol (hydroxypyrazolo(3,4-d)pyrimidine) (HPP) is by far the most promising drug now available for treatment of gout and

related hyperuricemic disorders. It represents a new and rational approach to therapy by inhibiting an enzyme essential to uric acid biosynthesis.

The correlation between gout and clinical manifestations of atherosclerosis associated with hypercholesterolemia and hypertriglyceridemia dictates that drugs used in treating hyperuricemic conditions should not elevate serum lipids. A further elevation of these parameters could increase the incidence of cardiovascular disease in highly susceptible gouty subjects.

A possible causal relationship between therapeutic use of allopurinol and elevations in blood lipid values (human subject) was reported for the first time by this station in April, 1969 (1). A subsequent preliminary animal study indicated an apparent experimentally provoked hypercholesterolemic hyperlipemia in rabbits administered allopurinol (1). This study was initiated to examine the problem in greater detail.

LITERATURE REVIEW

Gout

History

Gout or podagra was one of the earliest disease entities described in medical literature. Although first use of the word "gout" occurred in the 13th century accurate descriptions of the disease were recorded by Hippocrates as early as the 5th century B.C. He noted specific familial, age and sexual distribution patterns and described the classic pathophysiology and symptomatology of acute gouty attacks in great detail. Empirical treatment of acute attacks of gout employing colchicine was advocated by Alexander of Tralles in the sixth century A.D. The diagnostically specific response of acute gouty attacks to colchicine has helped to distinguish it from otherwise similar clinical entities.

Until very recently ignorance of the basic metabolic mechanisms pertinent to gout has limited our understanding of the disease and made rational treatment impossible. Progress was made primarily in improvement of diagnostic procedures and in answering questions concerned with heredity and pathophysiology of hyperuricemia in acute gouty attacks. Several reviews on the history, early diagnosis and treatment of gout are available (2); (3); (4).

Serum Uric Acid Concentration

Research efforts over the past thirty years have resulted in elucidation of a multiplicity of mechanisms which may result in hyperuricemia and its apparent clinical sequela.

Jacobson (5) has suggested arbitrary normal limits for uric acid of 7.0 mg/100 ml of serum for males and 6.0 mg/100 ml for females based on an extensive population survey. Seegmiller et al. (6) have shown an appreciable overlap of distributions in nongouty and gouty subjects between 6.0 - 7.5 mg/100 ml. Therefore, Stecher et al. (7) have proposed that emphasis be placed upon probability of development of gout as predicted by serum uric acid values, and not upon arbitrary division of "normal" and "abnormal" levels.

Gout is a disease entity peculiar to man. Lack of hepatic uricase in man may play a role in augmenting gouty symptomatology but is not entirely responsible for the disease syndrome since only a small fraction of the population is affected. In most mammalian species uricase oxidizes uric acid, which is only sparingly soluble, to allantoin. This metabolic end product is quite soluble in body fluids and exhibits a high renal clearance.

Hereditary Factors In Hyperuricemia

Impressive and extensive evidence is available which points to a pattern of hereditary control of serum uric acid levels in families of gouty subjects and isolate ethnic groups.

Genetic studies of hyperuricemia in families of gouty patients led Smyth et al. (8) to conclude that hyperuricemia resulted from an autosomal dominant gene of low penetrance in females. This hypothesis helped to explain why mean concentrations of urate in serum were found to be appreciably higher in male members of families having gouty patients (9). However, Talbott (10) found the incidence

of hyperuricemia in genetically prone families variable and suggested that a polygenic trait was responsible.

Absolute genetic control of familial tendencies has not been proven but impressive evidence published by O'Brien et al. (11) pointed to autosomal dominant polygenic factors being responsible for high positive correlations noted between serum uric acid levels and both weight and body surface area. Shyam et al. (12) reported significantly higher levels of uric acid in serum of patients with Down's syndrome in all age groups and in both sexes when compared to control subjects. No differences were noted in patients with Klinefelters syndrome or in parents of mongoloids.

Becker and Wallin (13) have recently described a true congenital hyperuricosuria syndrome consisting of choreoathetosis, mental retardation, hyperuricemia and radiologic changes related to cerebral palsy. The condition is seen only in males and is inherited as a sex-linked recessive trait.

Unusually high concentrations of urate in serum have been noted in the Chamorros of the Mariana Islands by Burch et al. (14), in Filipinos by Decker et al. (15), in the Maori of New Zealand by D'Lennane et al. (16) and in the Blackfeet and Pima Indians of the United States by O'Brien et al. (11).

The discovery that mean serum uric acid levels of certain ethnic and reginal subgroups differ significantly from population means is further complicated by similar correlations with other nonhereditary factors. Rasch et al. (17) have shown an unexplained association

between individuals with elevated serum uric acid levels and above-average intelligence, achievement (military motivation), and/or athletic ability.

It is abundantly clear that statistical definition of an exact normal serum uric acid level or value to sharply demarcate normal from abnormal values would, at best, be an approximation.

Wyngaarden (18) has suggested that theoretical sites of regulatory defects of potential importance in the pathogenesis of hyperuricemia are numerous and include both multiple genetic and nonhereditary factors important in ultimate control of purine biosynthesis and uric acid excretion.

Uric Acid Biosynthesis and Metabolism In Gout

Garrod (19) first documented the relationship between hyperuricemia and gout. A multiplicity of regulatory defects may lead to hyperuricemia and subsequent gout. However, defective purine metabolism is considered the primary biological mechanism in its pathogenesis. Irrespective of etiology, deposition of uric acid in body tissues results in clinical manifestations of gout.

Wyngaarden (20) has reviewed, in depth, the de novo pathway of purine synthesis and outlined less important pathways of purine nucleotide interconversion and of oxypurine and uric acid formation in explaining potential pathophysiologic mechanisms of hyperuricemia in gout. Details of individual reaction sequences may be found in reviews by Hartman et al. (21) and Buchanan (22). Reichard et al. (23) have postulated complex feedback mechanisms, called "Salvage

Pathways" which may serve to regulate purine biosynthesis in certain gouty patients. Salvage pathways would contribute to excessive de novo purine synthesis, and resulting hyperuricemia, if adenine and quanine were to react with 5-phospho-D-ribosyl-1-pyrophosphate to form the corresponding ribonucleotide directly.

Hyperuricemia results from multiple causal factors. Dietary intake of purines contributes to uric acid formation but rates of endogenous de novo biosynthesis and/or catabolism of cellular nucleoprotein with resulting oxidation of purine ribosides to uric acid are considered primarily responsible for regulating plasma urate levels. Metabolic degradation and conversion of purine substances to uric acid is outlined in Figure !.

Disposition of Uric Acid In Gout

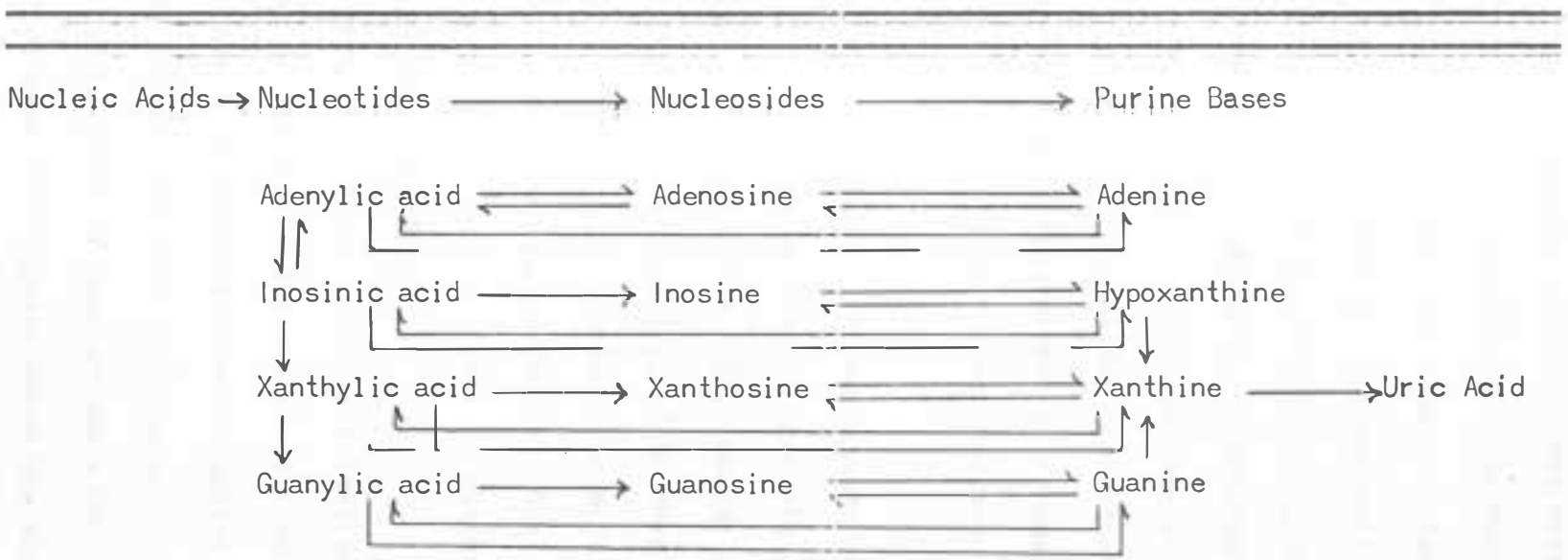
Extracellular fluids in most mammals supersaturate with uric acid at concentrations above 6.4 mg/100 ml. Although supersaturated solutions may exist without crystal formation the normal level of diffusible (miscible) uric acid in the extracellular fluid pool is about one gram whereas in tophaceous gout levels may exceed 35 grams.

Hyperuricemic individuals establish a dynamic equlibrium between plasma diffusible (miscible) and tissue tophaceous (immiscible) urate pools. Therefore, serum uric acid levels below 6.0 mg% will generally result in dissolution of tophaceous deposits and serum levels above this amount in deposition of urate crystals.

Urinary uric acid excretion in adult human males on purine-free diets ranges between 300 to 600 mg per day (endogenous biosynthesis).

FIGURE I

METABOLIC DEGRADATION AND CONVERSION OF PURINE SUBSTANCES



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Data of Seegmiller et al. (24) indicate urinary uric acid excretion represents over 75% of total body urate turnover. Extrarenal mechanisms account for the remainder. Gastrointestinal uricolysis resulting from action of bacterial flora accounts for disposal of approximately 200 mg of uric acid daily yielding urea, ammonia and carbon dioxide as primary end products. Effective bacterial degradation prevents appearance of uric acid in stools unless antibiotic sterilization of the gut or intestinal bacteriostasis has occurred.

Measurable but insignificant extrarenal uricolysis in mammalian tissue due to verdoperoxidase has been reported by Cannellakis et al. (25)..

Theories concerning renal mechanisms responsible for uric acid excretion based on the premise of complete glomerular filtration followed by highly effective (95%) tubular reabsorption of urate are no longer tenable. Gutman et al. (26) have strong evidence to suggest complete reabsorption of filtered uric acid in proximal convoluted tubules. Their evidence also suggests that excreted uric acid is produced entirely by active distal secretion.

Primary and Secondary Gout

Symptoms of gout are common clinical sequela of hyperuricemia regardless of etiology. Gout is classified clinically as "primary" or "secondary".. A classification of varieties of gout according to Wyngaarden (27) is outlined in Table I.

The pathogenesis of hyperuricemia in primary gout is independent of diverse disease states which may elevate plasma urate levels

TABLE I

A CLASSIFICATION OF VARIETIES OF GOUT

Primary gout
Familial
Nonfamilial
Secondary gout
Hematologic disorders
Myeloproliferative diseases
Hemolytic diseases
Hyperuricacidemic drugs
Obesity
Starvation
Chronic renal disease
Glomerulonephritis and pyelonephritis
Saturnism
Hypertensive cardiovascular disease
Glycogen storage disease, Type I
Juvenile gout, choreoathetosis, and mental retardation
Miscellaneous

Reproduced from: Advances in Metabolic Disorders. Vol. II, p. 56. 1965.

and has been attributed to both overproduction of purines and underexcretion of uric acid or both.

Talbott (28) has shown primary gout highly familial in distribution. A nonfamilial type of primary gout occurs occasionally in individuals from normouricemic families. Apparently rare cases of nonfamilial primary gout result from disturbances in nonhereditary mechanisms involved in uric acid homeostasis.

Secondary gout occurs when a disease or condition, excluding hereditary predisposition, is considered directly responsible for appearance of predisposing hyperuricemia. In these patients subsequent clinical symptoms of gout have been shown to follow logically upon defects or conditions such as administration of hyperuricacidemic drugs (29), obesity (30), starvation (31), chronic renal insufficiency (32), hyperparathyroidism (33), psoriasis (34), glycogen storage disease (35), sarcoidosis (36), polycythemia vera (37), multiple myeloma (38) and leukemia or other hematopoietic system diseases (39).

Clinical incidence of secondary hyperuricemia has increased markedly since the introduction of antitumor agents which result in rapid resolution of large tissue masses with subsequent catabolism of cellular nucleoprotein to uric acid. Patients suffering from secondary gout differ from individuals with the primary form in several ways. Secondary hyperuricemia results in more severe vascular nephrosclerosis and a higher incidence of renal calculi apparently the result of increased uric acid excretion. Females show

a higher incidence of the secondary form than do males and tophaceous deposits are more widespread and numerous.

Published data have shown that patients suffering from primary gout exhibit a markedly increased total body uric acid pool in the absence of pathologic cellular breakdown. Therefore, Yu et al. (40), on the basis of isotope incorporation studies, have suggested that primary gout results from substantially increased de novo synthesis of purines and uric acid.

Uric Acid and Lipid Metabolism

It is well recognized that hyperlipidemia and hyperuricemia occur in a high percentage of patients suffering from both gout and showing clinical manifestations of atherosclerosis and ischemic heart disease (41); (42).

Analysis of data from the Framingham population survey (43) indicated that gouty individuals had a frequency of coronary artery disease twice that of nongouty subjects. Available evidence at that time pointed to a causal relationship between clinical atherosclerosis in gout and hypercholesterolemia (44).

Traut (45) demonstrated urate deposits in both organized thrombi and in proliferated arterial intima. Kohn and Prozan (46) suggested that urates acted as a surfactant to promote subintimal lipid deposition resulting in occlusive arterial disease and atherosclerosis. Subsequent studies suggested a more precise association between hyperuricemia and hypertriglyceridemia (47) and later studies by Fasoli et al. (48) pointed to elevated serum B-lipoprotein levels.

Recent findings of Barlow (49) do not lend support to current views which hold triglycerides more important than cholesterol in any association with uric acid levels. His data show hypertriglyceridemia is not found more frequently than hypercholesterolemia in gout when adequate control groups of both normo- and hyperlipidemic subjects are employed and effects of age and sex on serum lipid levels are considered. In addition, he states that it does not appear justifiable to explain hyperlipidemia in gouty subjects merely as an expression of atherosclerosis but suggests instead a metabolic and/or genetic association between observed changes in serum concentrations of lipid and urate in gouty individuals which involves both cholesterol, triglycerides and other contributory factors such as hypertension, obesity and glucose intolerance.

Pathogenesis of Primary Gout

Gout is commonly attributed to both overproduction of purines and underexcretion of uric acid. Sorensen (32) has coined the terms "primary metabolic gout" and "primary renal gout" to describe individuals who appear to represent pure examples of either overproduction or underexcretion of uric acid, respectively. However, Seegmiller et al. (50), in both group analyses and studies of individual case histories, have shown that the vast majority of gouty subjects exhibit evidence of both abnormalities.

Garrod (19), in 1876, first suggested that acute gouty attacks resulted from an inflammatory response to sodium urate salts deposited in tissues. It is now generally accepted that gout is a crystal

deposition disease. However, it was not until the work of McCarty et al. (51) was published in 1961 that a logical explanation was available to explain acute inflammatory symptoms. They viewed the acute gouty attack as involving a number of crystal depositions and host responses. The inflammatory response was shown to be dependent on phagocytosis of microcrystalline sodium urate by leukocytes (neutrophiles) which ultimately lyse freeing certain kinin peptides. These peptides ultimately cause pain, vasodilatation, local necrosis and fibrotic proliferation which result in formation of subcutaneous tophi.

The tophaceous nodule is a pathognomonic lesion in classical gout. It is composed of multicentric deposits of amorphous or crystalline urates arranged radially in a matrix composed of a polysaccharide material, lipid (cholesterol), protein and occasionally calcium (52). Howell et al. (53) have shown both non-palpable tissue deposits and tophi to be monosodium urate monohydrate. Bartels and Matossian (54) have reported a definite correlation between tophi formation and height of serum uric acid levels (above 8.0 mg%), degree and frequency of renal damage and duration of clinical symptomatology.

The solubility of monosodium urate appears reduced in sites predisposed toward tophaceous deposits. These include articular and ear cartilages, synovial tissue, epiphyseal bone and heart valves.

Tissue pH may play an important role in tophi formation since acidic media favors urate precipitation. Relatively avascular areas

such as the ears or toes tend to be "acidic" because of their reduced temperatures, low oxygen tension and dependence on glycolysis, with resulting lactic acid formation, for energy production.

Renal pathology is common in patients suffering from gout and is the eventual cause of death in 25% of these individuals. Sokoloff (52) has published data which extend and strenghthen the traditional view that renal lesions result from precipitation of urates with obstruction of collecting tubules, and secondary necrosis, fibrosis and atrophy of proximal convoluted tubules. A typical and distinctive glomerulosclerosis may be a reaction of the kidney to increased filtration of uric acid. Renal damage in gout is said to hinge more upon uric acid solubility in tubular fluid and urine than its solubility in plasma.

Diagnosis and Treatment of Gout

Diagnosis of gout is accomplished by careful analysis and evaluation of (1) clinical symptomatology (2) additional early supporting evidence (3) appearance of later developing diagnostic criteria and (4) positive laboratory and/or radiographic findings.

Gout should always be considered in cases of acute, unexplained articular distress in postmenopausal women or previously healthy men. Classic clinical findings of the acute attack are usually sufficient to establish a diagnosis in gout. A history of familial hyperuricemia is particularly useful in making an early differential diagnosis between acute articular gout, chondrocalcinosis (pseudogout) and rheumatoid arthritis. Although changes in serum uric

acid levels are not considered causal in precipitation of acute attacks hyperuricemia is present in all but 5% of gouty subjects and is considered a valuable diagnostic aid. The presence of urate crystals in synovial fluid aspirated from an inflamed joint during an acute attack or from tophi are absolute and reliable diagnostic criteria for gout.

Colchicine is so dramatic and selective in its ability to alleviate joint pain in acute gouty attacks it is used as a diagnostic tool. However, the test is not infallible since occasional gouty subjects fail to respond and false positives occur in about 5% of patients suffering from nongouty arthritic disorders.

Radiographic changes characteristic of cystic joint, cartilage and epiphyseal bone destruction, caused by replacement of calcium salts by monosodium urate, are common in chronic gout.

The most common clinical symptoms and laboratory findings noted in primary gout are listed in Table II.

Colchicine

Colchicine is an alkaloid of Colchicum autumnale (meadow saffron). Although the drug was not isolated by Pelletier and Caventou from colchicum until 1820, crude plant preparations were prescribed for pain of articular origin as early as the sixth century by Alexander of Tralles. Colchicine will dramatically and specifically terminate an acute gouty attack. It does not have analgesic or uricosuric properties and is not capable of altering either serum uric acid levels or amounts of diffusible (miscible) uric acid in

TABLE II

CLINICAL SYMPTOMS AND LABORATORY FINDINGS NOTED IN PRIMARY GOUT

ARTICULAR SYMPTOMS
- Acute articular pain
- Onset sudden, severe and often nocturnal
- Usually monoarticular involvement
  - Metatarsophalangeal joint of great toe
  - Ankle
  - Knee
  - Wrist
- Involved joints tender, swollen, hyperemic
- Pronounced limitation of motion (ankylosis)
- Cystic changes in epiphyses of bone
- Joint and cartilage destruction
- Presence of urate crystals in synovial fluid
- Acute episodes followed by periods of complete remission

EXTRAARTICULAR COMPLICATIONS (RENAL INVOLVEMENT)
- Albuminuria
- Uric acid urolithiasis
- Arteriolar nephrosclerosis
- Glomerulonephritis
- Pyelonephritis
- Delayed phenolsulfonphthalein excretion

SYSTEMIC REACTIONS
- Moderate fever
- Chills
- Headache
- Tachycardia
- Malaise
- Anorexia
- Leukocytosis
- Hyperuricemia
- Presence of subcutaneous tophi

the total body pool. The exact mechanism by which it exerts its anti-inflammatory action is still unknown and remains as one of the oldest mysteries in medicine.

Several theories have been proposed in an attempt to explain the anti-inflammatory action of colchicine. Seegmiller et al. (55) and Howell et al. (56) have suggested that the drug inhibits leukocyte metabolism and subsequent elevations of lactic acid which would otherwise occur as a result of urate crystal phagocytosis during an acute gouty attack. Malawista (57) has suggested that colchicine suppresses pinocytosis in neutrophiles making phagocytosis of urate crystals impossible. Such a mechanism would effectively block the ability of affected leukocytes to participate in the inflammatory response.

Although the exact biochemical step or steps influenced by colchicine are unknown they are independent of antimitotic activity.

The total amount of colchicine usually required to terminate an acute gouty attack ranges between 4.0 to 8.0 mg. An initial dose of 0.5 to 1.0 mg is followed by 0.5 mg doses each hour until resolution of the attack occurs or toxic symptoms ensue.

Uricosuric Agents

Uricosuric drugs are, in essence, urate diuretics. Described as a group they are organic acids which compete with sodium urate for anionic transport sites in the nephron. The therapeutic objective of uricosuric drug administration in gout is reduction of diffusible (miscible) uric acid in the extracellular fluid pool.

Clinically, Gutman and Yu (58) have shown dramatic success in resolution of subcutaneous tophi in gouty subjects when serum urate levels were lowered to 6.0 mg% by administration of uricosuric drugs. The two most commonly employed drugs in this class are probenecid (Benemid) and sulfinpyrazone (Anturane).

Probenecid was one of a number of drugs developed by Beyer and his associates (59) in an extensive and systematic study to find a compound that would effectively inhibit the rapid renal excretion of penicillin. Although probenecid will effectively inhibit rapid excretion of penicillin it is now used clinically almost exclusively for its uricosuric properties.

Probenecid, U.S.P. (Benemid) is available commercially in 0.5 gm scored tablets. In the treatment of chronic tophaceous gout 0.25 gm of the drug is administered twice daily for one week, initially, and 0.5 gm twice daily thereafter.

Burns et al. (60) described the uricosuric activity of sulfinpyrazone, a sulfoxide metabolite of phenylbutazone, in 1958. Although sulfinpyrazone (Anturane) lacks the analgesic and anti-inflammatory properties of phenylbutazone it retains the potent uricosuric activity of its congener. Gutman (61) has published a detailed description of sulfinpyrazone therapy for chronic gout.

Opinions regarding indications for uricosuric therapy vary considerably. The only unequivocal indication for their use appears to be in tophaceous gout. Most authorities do not consider asymptomatic hyperuricemia an indication for therapy since evidence to

show that uricosurics either ameliorate or prevent renal damage in gout is lacking.

Inhibitors of Uric Acid Synthesis

Ideally, rational treatment of gout would involve pharmacological correction or amelioration of basic cellular metabolic defects which result in hyperuricemia and its ultimate clinical sequela. At the present time rational treatment is aimed at inhibition of accelerated purine synthesis and uric acid formation known to occur in gouty subjects.

Orotic Acid

Fallon et al. (62) have shown that large doses of orotic acid (15 gm/day) will result in uricosuria and clinical improvement in gouty subjects. The mechanism of action of orotic acid is unknown. However, Delbarre and Auscher (63) have evidence to suggest that it inhibits de novo purine biosynthesis.

Clinical use of the compound is extremely limited because of a high incidence of adverse side effects. Huguley et al. (64) have reported multiple cases of renal urolithiasis and Creasey et al. (65) have evidence to suggest a causal relationship between orotic acid administration and fatty infiltration of the liver in laboratory animals.

L-Glutamine Analogs

Two structural analogs of L-glutamine, 6-diazo-5-oxynorleucine (DON) and azaserine, have been shown to effectively inhibit glycine-1-^{14}C incorporation into uric acid in man and result in reduction of

both serum and urinary urate levels (66); (67). Although these compounds are useful research tools they cannot be employed clinically because both are highly toxic to buccal and gastrointestinal mucosa.

Allopurinol (4-Hydroxypyrazolo(3,4-d)pyrimidine)

Allopurinol (Zyloprim) has found extensive clinical use in treatment of hyperuricemia and hyperuricosuria of diverse etiology. Klinenberg *et al.* (68) have described the drug as a new and truly rational approach to therapy in gout and related hyperuricemic conditions.

Allopurinol is a close structural analogue of hypoxanthine and a potent inhibitor of xanthine oxidase which has been demonstrated necessary for conversion of hypoxanthine and xanthine to uric acid *in vivo*. Antihyperuricemic activity follows blockade of this essential step and results in reduction of both serum and urinary uric acid levels by inhibition of *de novo* synthesis, not by increased urinary loss, as with uricosuric agents.

Available evidence indicates allopurinol does not disrupt biosynthesis of vital purines and inhibits only those biochemical steps in purine catabolism which immediately precede uric acid formation.

Chemistry

Xanthine oxidase is responsible for catabolism (inactivation) of 6-mercaptopurine (6-MP) and related thiopurines used in cancer chemotherapy. A structural analogue of the purine base hypoxanthine, 4-hydroxyprazolo(3,4-d)pyrimidine, was originally one of a number of purine analogues, that act as xanthine oxidase inhibitors *in vitro*, screened by Hitchings and Elion (69) for potential use as an adjuvant in anti-neoplastic disease therapy. Their aim was to increase therapeutic efficacy of purine analogs such as 6-MP by slowing rates of

metabolic inactivation by inhibition of xanthine oxidase. Elion (70) chose 4-hydroxypyrazolo(3,4-d)pyrimidine for *in vivo* studies after analysis of preliminary data indicated that it was relatively non-toxic. The compound was initially referred to as "B.W. 56-158" (Wellcome Research Laboratory), later as "HPP", and has now been given the generic name allopurinol. Falco *et al.* (71) synthesized the pyrozolo(3,4-d)pyrimidine series from which the compound was obtained in 1956.

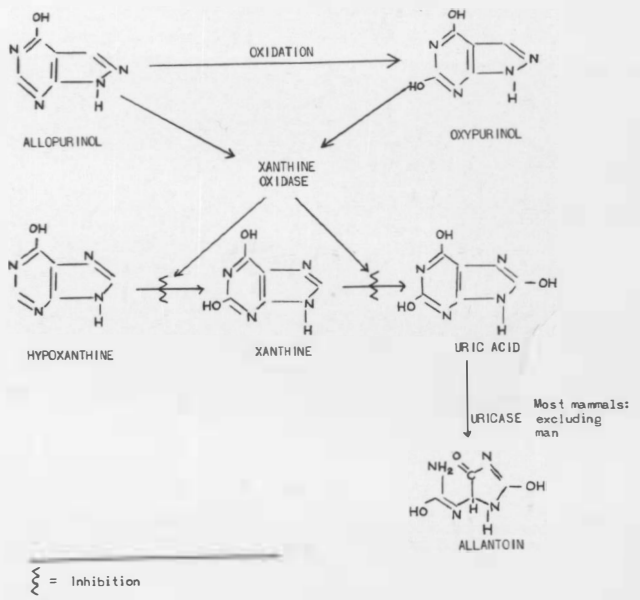
Allopurinol is a structural analogue of the natural purine base, hypoxanthine, differing by transposition of carbon and nitrogen atoms in the seven and eight positions (Fig. 11). It is both a potent inhibitor of, and a substrate for, xanthine oxidase (72). The compound acts as a competitive inhibitor of the enzyme at low inhibitor levels (73) and a noncompetitive inhibitor at higher concentrations (74). The product of its xanthine oxidase-catalyzed oxidation is a xanthine analogue, oxypurinol or alloxanthine, 4,6-dihydroxypyrazolo(3,4-d)pyrimidine, which is also an inhibitor of xanthine oxidase (75); (76).

Available evidence indicates that neither allopurinol nor oxypurinol exhibits binding to plasma proteins and that tissue and organ concentrations do not differ from equal distribution in total body water, with the exception of brain which exhibited about half the amounts isolated from other tissues (75).

Taggart (77) has shown that oxypurinol is not a member of the "organic acid system" and Weiner *et al.* (78) have published data

FIGURE II

CHEMICAL STRUCTURES AND RELATIONSHIPS BETWEEN ALLOPURINOL, OXYPURINOL AND TERMINAL COMPOUNDS IN THE BIOSYNTHESIS OF URIC ACID



Reproduced from: Thibodeau et al., S. Dak. J. Med. 22:22, 1969.

which seperates it, chemically, from uricosuric drugs. Elion *et al.* (75) have suggested that oxypurinol appears more as an analogue of uric acid than xanthine when considering functional groups in the pyrimidine ring or the presence of an unshared electron pair in the region of the purine-8 position. Their data suggest that this unshared electron pair may be the structural feature which determines oxypurinol behavior.

Pharmacology

Pharmacologic effects of allopurinol administration are, almost without exception, directly related to its blockade of terminal biochemical reactions in *de novo* uric acid biosynthesis via xanthine oxidase inhibition.

Allopurinol and its *in vivo* oxidative metabolite, oxypurinol (alloxanthine), are both potent inhibitors of xanthine oxidase (75). Therefore, pharmacologic half-life of effective xanthine oxidase inhibition is dependent upon both renal clearance of administered allopurinol and rate of oxidation to, and subsequent excretion of, oxypurinol. Oxypurinol has been shown to be a less effective inhibitor than allopurinol (79) but is of considerable pharmacologic importance because of its longer half-life. Accumulation occurs as a consequence of low renal clearance. Further, direct inactivation of xanthine oxidase by both allopurinol and oxypurinol influence ultimate clinical responses. Rundles *et al.* (80) have shown that endogenous synthesis of xanthine oxidase is neither induced by treatment with allopurinol nor inhibited because of the subnormal activity

maintained after its administration.

Efficacy of allopurinol as an *in vivo* xanthine oxidase inhibitor was first confirmed in results published by Rundles *et al.* (81) in 1963. They noted a marked reduction in dosage requirements of sulfur-containing hypoxanthine analogues (6-MP) needed for adequate therapeutic response in cancer chemotherapy if the compounds were administered concurrently with allopurinol. Enhancement of 6-MP activity was correctly attributed by Elion *et al.* (82) to *in vivo* suppression of xanthine oxidase activity preventing oxidation of 6-MP to its inactive 6-thiouric metabolite. In addition they noted striking reductions in serum and urinary uric acid levels not observed with administration of 6-MP alone. White (83) has shown allopurinol completely ineffectual when administered alone as an antineoplastic drug. Subsequent data published by Shaw *et al.* (84) extended and corroborated these findings.

A consistent dose-dependent reduction in both serum and urinary uric acid levels has been shown to occur following initiation of allopurinol administration in gouty subjects (85).

Dosage levels of allopurinol needed to reduce hyperuricemic blood levels to normal ranges vary from 400 to 600 mg per day in severe tophaceous gout to 200 to 300 mg per day, in divided doses, for subjects with mild forms of the disease. Serum urate levels fall quite slowly after initiation of therapy and increase to hyperuricemic levels only gradually after drug withdrawl because of prolonged oxypurinol half-life (slow renal clearance).

Recent data published by Elion et al. (75) suggested augmented renal clearance of oxypurinol during uricosuric drug therapy. They noted a definite decrease in xanthine oxidase inhibition by oxypurinol during combined therapy but often found such treatment effective in achieving minimal serum urate levels in gouty patients with nephropathy. Klinenberg et al. (86) found allopurinol used in conjunction with uricosuric drugs allowed maximal total purine excretion. Yu and Gutman (85) reported allopurinol capable of reducing plasma urate levels in the presence of gouty nephropathy sufficient to render uricosuric drugs ineffective.

Results of renal studies including glomerular filtration rate and urate clearance in patients administered allopurinol show no significant differences when compared to results obtained prior to initiation of therapy.

Data published by Klinenberg et al. (86) also suggest that allopurinol administration which results in excretion of three (rather than one) purine metabolic end products, each with its independent solubility level, provides a more favorable condition for elimination of relatively insoluble uric acid. Danger of urinary calculi formation diminishes further as urate excretion is reduced during therapy. Amounts of allopurinol and oxypurinol excreted are well within limits of their respective solubilities if administered at recommended dosage levels.

Toxicology

Although specific studies of possible diverse function have

not been published, xanthine oxidase is known to be a versatile enzyme capable of acting on a variety of substrates (87). Therefore, *in vivo* inhibition following allopurinol administration could theoretically precipitate multiple clinical complications. In all probability, however, therapeutic dosage levels of allopurinol do not completely inhibit *in vivo* xanthine oxidase activity. Further, xanthinuric subjects who show a complete lack of xanthine oxidase activity from birth live in comparatively good health.

There is now a wealth of experimental data, from both animal and clinical research endeavors to substantiate initial reports describing allopurinol, in general, as an exceptionally well tolerated drug (88); (89); (90).

Results of early clinical studies by Yu *et al.* (85), and others, listed an increase in number of acute gouty attacks during initial stages of allopurinol therapy as the most troublesome complication encountered. Although attacks usually became shorter and decreased in severity as therapy continued, prophylactic administration of colchicine is now recommended when allopurinol therapy is initiated.

In general, drug idiosyncrasy has not constituted a serious deterrent to allopurinol administration. Most drug reactions attributed to allopurinol, including fever, chills, pruritus, nausea and vomiting have been minor and transient.

Severe reactions, although rare, have been reported and include impaired renal function (88), and leukopenia (86). Adverse effects on hepatic iron storage (91); (92) and impaired iodine metabolism

(93) have been reported following allopurinol administration. Pinnas (94) has reported a possible association between macular lesions and allopurinol and Thibodeau et al. (1) have suggested a possible causal relationship between its use and subsequent hyperlipemia.

The LD_{50} for allopurinol in mice is reported to be 160 mg/kg i.p. and 700 mg/kg p.o. The acute LD_{50} in rats is listed as 750 mg/kg i.p. and 6000 mg/kg p.o. (95).

Teratogenic and reproductive studies with both rabbits and rats showed allopurinol administration had no effect on litter size, number of stillbirths or malformations. Mean weights of young at birth and at three weeks postpartum were not affected (95).

Indications for Use

Absolute and definite clinical indications for therapeutic and/or prophylactic use of allopurinol either alone or in combination with uricosuric agents, colchicine, phenylbutazone (Butazolidin), corticosteroids or synthetic nonsteroidal anti-inflammatory agents such as indomethacin are not yet clearly formulated.

Allopurinol is not an innocuous drug. Although studies to date have shown it to be remarkably well tolerated by most subjects long term effects of administration are not known. Therefore, strict attention should be given to recommended indications for its use and until more definitive investigations are completed indiscriminate clinical use in other hyperuricemic states is contraindicated.

Table III lists presently recommended indications for allopurinol administration. It should be noted that pediatric use is

TABLE III

RECOMMENDED INDICATIONS FOR ALLOPURINOL ADMINISTRATION

Familial or nonfamilial primary hyperuricemia (gout)

Secondary hyperuricemia (gout) due to:

- Myeloproliferative disease
- Polycythemia vera
- Leukemias
- Radiation therapy or antineoplastic drug use

Primary uric acid nephropathy

Prophylaxis of urate nephropathy in neoplastic disease states

Uric acid urolithiasis and tissue urate deposition in gouty arthritis

Concomitant use with antineoplastic drugs to reduce required dosages

Concomitant use with uricosurics to obtain maximal purine excretion

Control of uric acid urolithiasis in patients with hyperuricosuria

Prevention of acute renal failure secondary to urolithiasis

contraindicated except in cases of secondary hyperuricemia in children with confirmed malignancy. Available teratogenic and reproductive studies employing laboratory animals have shown allopurinol free of adverse effects. However, the effects of xanthine oxidase inhibition on the human fetus are unknown. Therefore, allopurinol should be used in pregnant women or women of childbearing age only if potential patient benefits are weighed against possible fetal risk. Individuals who show severe reactions such as hepatotoxicity, impaired renal function or leukopenia following initial allopurinol administration should not be restarted on the drug.

EXPERIMENTAL METHOD

Experimental Animals

Sixteen purebred, Dutch Black and Grey, virgin doe rabbits weighing 1435 to 2114 grams were utilized in this investigation. All animals received an extensive initial clinical examination and were found alert, active and apparently disease-free.

Experimental animals were individually housed in stainless steel batteries and maintained on a commercially available pelletized rabbit diet[1] and mineral supplement[2]. Feed and water were provided ad libitum. Animal quarters were air conditioned to minimize environmental temperature fluctuations.

Treatment and Control Grouns

Following a two-week adaptation period a table of random numbers was employed to assign animals to either experimental or control groups each containing six does. Experimental animals received 50 mg of commercially available allopurinol suspended in one ml of distilled water, orally, daily for 14 days. Control group does received an equal amount of Normal Saline for a similar period employing identical techniques.

Drug Preparation: Oral Administration

Allopurinol employed in this study was obtained commercially in tablet form. Tablets were crushed in a mortar and pestle and the

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[1]Rabbit Family Ration. Albers Milling Company. Los Angeles, Calif.

[2]Carey Mineral Supplement Spools with Salt. Carey Salt Co.

finely ground powder suspended evenly in water immediately prior to administration. Highly alkaline diluent, which might have resulted in gastric irritation, would have been necessary for compounding a true drug solution. Problems commonly associated with repeated passage of gavage tubes were eliminated by use of a tuberculin syringe for oral drug administration employing a technique previously described (96).

Collection of Data

Animals were weighed when assigned to experimental or control groups, then isolated from other experimental animals for seven days prior to initiation of the 14-day treatment period. Animals were weighed a total of five times:

1. Seven days prior to initiation of treatment (Day 1)
2. The first day of drug administration (Day 7)
3. The seventh day of drug administration (Day 14)
4. The 14th day of drug administration (Day 21)
5. Seven days post-treatment (Day 28).

Blood was drawn for analysis of lipid, uric acid and hemogram values on days 7, 14, 21, and 28. Lipid values monitored were total cholesterol and triglycerides; hemogram values included packed cell volume and total hemoglobin.

Unanesthetized rabbits were suppinated and immobilized on a slatted small animal holding board. The thorax was shaved and painted with an aqueous solution of 1:750 Zepharin Chloride before 10 ml of blood was drawn by cardiac puncture employing aseptic techniques.

A 20 gauge needle was used to minimize hemolysis and sodium heparin was employed as an anticoagulant.

Packed cell volume and hemoglobin values were determined immediately. Whole blood was centrifuged, plasma decanted and then quick frozen for analysis of lipid and uric acid levels at a later date.

Hemoglobin and Packed Cell Volume Determinations

Measurement of total hemoglobin is a routine and commonly employed technique in clinical laboratory medicine. The Cyanmethemoglobin Method is recommended as superior by the Clinical Pathology Standards Program of the College of American Pathologists and was employed in this study (97).

Cyanmethemoglobin color complexes are remarkably stable and possess excellent spectral characteristics. A linear five-point standard curve, blank, 5, 10, 15 and 20 grams percent hemoglobin, was prepared employing a commercially available cyanmethemoglobin[3]. Absorbance in optical density figures or percent transmission of the standard was found to be equivalent to 20 gm% hemoglobin when 0.02 ml of blood was added to 5.0 ml of a reagent supplied by the same company. A Beckman Model DB-G spectrophotometer set at 540 mμ was employed.

Determination of total hemoglobin in unknown samples was accomplished by adding exactly 0.02 ml of blood to 5.0 ml of reagent,

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[3] Hycel Cyanmethemoglobin Determinations. Hycel, Inc. Houston, Texas

inverting several times, and reading duplicates against a Reagent Blank at 540 mμ. Readings were transferred to a standard curve and total hemoglobin concentration in gm% obtained directly. Total hemoglobin measured by this method included oxyhemoglobin, carboxy-hemoglobin and methemoglobin and was found accurate in practice to 3% variation. The only case in which cyanmethemoglobin would not represent total hemoglobin is in sulfhemoglobinemia (97).

The cyanmethemoglobin reagent employed effected a rapid hemolysis of erythrocytes and formation of a stable color complex. Microscopic examination of the reagent-blood mixture verified complete hemolysis indicating an absence of certain abnormal cells known to be resistant to lysing in this media. Cells resistant to hemolysis are associated with hemolytic and sickle cell anemias and traits, thalassemia major and minor, hepatocellular jaundice, hemoglobin C disease and cases of reversed albumin-globulin ratios.

Packed cell volume values were obtained employing standard micro-techniques. Experimental blood samples were well mixed and introduced into heparinized microhematocrit capillary tubes. Tubes centrifuged at 3000 rpm for five minutes showed distinct separation of erythrocytes from plasma. Values were obtained directly from scales imprinted on the specialized centrifuge base[4].

Determination of Total Cholesterol (Direct Method)

Cholesterol is soluble in many organic medias including ether,

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[4]Autocrit Centrifuge. Clay Adams Company.

chloroform, acetic anhydride and acetic acid. Differing but characteristic solubility patterns in these various media have led to many techniques for isolation and separation of cholesterol from whole blood and numerous blood fractions. The technique employed in this study is a modification of the method of Schoenheimer and Sperry (98).

A commercially available[5], stable, Lieberman-Burchard reagent was used for determination of total cholesterol in plasma. The reagent consists of 14.5% sulfuric acid in 41.0% acetic acid and 43.0% acetic anhydride. Reagent stability is apparently achieved by a process of compounding under controlled temperature conditions. Use of this reagent will result in a reaction specific for the double bond between C_5 and C_6 on ring B of the sterol molecule producing a typical green-blue color.

Timed sequence of reaction rates was not considered important since readings made within 10 minutes following color development were always stable. All samples were read within this time period. Absorbance characteristics of the reaction at 625 mμ followed Beer-Lambert's Law between 0 - 300 mg%.

A. Methodology

1. Accurately pipette 0.1 ml of plasma into marked cuvette. Place 0.1 ml cholesterol standard (200 mg%) into marked cuvette.

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[5] Hycel Cholesterol Reagent. Hycel, Inc. Houston, Texas

2. To each test tube or cuvette add 6.0 ml cholesterol reagent. Mix well by shaking.

3. Place cuvettes in water bath at 37 °C. for 20 minutes.

4. Read optical density against reagent at 625 mμ.

B. Calculations

1. "A" - Optical density of unknown

2. "B" - mg% value of control or standard

3. "C" - Optical density of standard or control

then; $\frac{A \times B}{C}$ = mg%, Unknown

Table IV indicates reproducibility of the Liebermann-Burchard color reaction employing Hycel stable cholesterol reagent.

Use of a stable reagent with a high specificity of reaction lends itself to a relatively simple methodology. However, the question of color development produced by blood constituents other than cholesterol is possible if bilirubin levels are highly elevated or considerable hemolysis is evident. Elevated bilirubin levels did not occur in either experimental or control animals used in this study and precautions were taken to minimize hemolysis when blood was collected. Adherence to accepted laboratory technique decreased the possibility of inadvertent transfer of red cells with the plasma sample or addition of moisture to the reaction. Such errors could falsely elevate apparent cholesterol values. If it had been necessary to use plasma showing significant hemolysis, values would have been rechecked using an alcoholic extract.

Specific for the double bond between C_5 and C_6 on ring B of the

TABLE IV

REPRODUCIBILITY OF THE LIEBERMANN-BURCHARD COLOR REACTION
EMPLOYING HYCEL STABLE CHOLESTEROL REAGENT

Concentration of Standard	Average	Std. Dev.	Range
100 mg% Standard	100.1 mg%	1.3 mg%	97 - 103 mg%
200 mg% Standard	199.8 mg%	2.3 mg%	195 - 205 mg%
300 mg% Standard	300.4 mg%	3.5 mg%	290 - 310 mg%

Recovery of Added Known

Added	Recovery
40 - 300 mg%	94 - 99 %

sterol molecule the reaction is not affected by uric acid, amino acids, vitamins, creatinine, creatine, protein, carbohydrates, or electrolytes. No agents presently known, including allopurinol, will depress the reaction.

Determination of Plasma Uric Acid Levels

Determination of uric acid levels in various body fluids is a routine procedure for most clinical and paramedical research laboratories. It is unfortunate that a majority of clinical (hospital) laboratories employ colorimetric procedures, of questionable accuracy, dependent entirely on formation of a blue-colored complex resulting from reduction of phosphotungstic acid by uric acid. Blanch and Koch (99) have shown that uric acid levels obtained from colorimetric procedures alone are almost always incorrectly elevated due to the presence of other blood constituents which yield similar color complexes.

The method of determining true uric acid levels employed in this investigation is a modification of an enzymatic procedure first described by Praetorius and Poulsen (100). All reagents needed for the procedure, including purified Uricase, are available commercially[6].

The underlying principle involved in the test is uricase enzyme specificity. Uric acid is the only purine oxidized by this enzyme

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[6]Sigma Chemical Co. St. Louis, Mo.

in a highly purified form (101).

Principle:

$$\underset{(\text{High } OD_{292})}{\text{Uric Acid}} + 2H_2O + O_2 \xrightarrow{\text{URICASE}} \underset{(\text{Low } OD_{292})}{\text{Allantoin}} + CO_2 + H_2O_2$$

Uric acid is measured by a decrease in optical density in a narrow band; wavelength close to 292 mμ (UV).

A. Methodology

1. Pipette into a test tube:

   0.2 ml Plasma

   1.0 ml Glycine Buffer

   6.0 ml distilled water (Mix well by shaking)

2. Pipette into each of two cuvettes 3.0 ml of mixture from Step (1) - Label one BLANK and the other TEST

3. Pipette into the BLANK:

   0.05 ml Uricase

   Allow both to stand at room temperature for 15 minutes.

4. a) Adjust spectrophotometer to wavelength of 292 mμ.

   b) Adjust slit width so that OD_{292} of BLANK reads 0.400.

   c) Read and record OD of TEST vs BLANK.

5. After approximately 5 minutes:

   a) Again adjust slit width so that BLANK reads 0.400.

   b) Read and record OD of TEST. If OD has not changed, this is considered as a "Final OD". If it has decreased, repeat readings at intervals until OD is constant. ("Final OD"). Time required is not critical.

B. Calculations

1. Since Uricase is not used in the BLANK, the small OD it contributes is approximately corrected for by adding 0.005 to the change in OD. (Sigma Uricase has been found to yield approximately this OD.)

2. $\Delta OD = (0.400 - \text{Final OD}) + 0.005$

$= (0.405 - \text{Final OD})$

3. Mg Uric Acid per 100 ml Plasma

$$= \frac{(3.05) \times (168.1) \times (100) \times (\Delta OD)}{(12{,}300) \times (0.0833)}$$

$= (50) \times (\Delta OD)$

C. Explanation of Factors Used in Calculations

1. 3.05 is the volume of Reaction Mix
2. 168.1 is the Molecular Weight of uric acid
3. 12,300 is the OD of 1 mole of uric acid in 1 liter
4. 100 converts 1 ml plasma to 100 ml.
5. 0.0833 is the volume of plasma used
6. 0.005 is the OD contributed by Uricase.

The OD of plasma at 292 mμ is very high. Extreme care was employed in pipetting to avoid introduction of significant error which could result if even small plasma volume changes should occur. Acetone will absorb light in the same wavelength as uric acid or allantoin. Therefore, cuvettes used in the procedure were air-dried without using acetone.

Determination of Plasma Triglyceride Levels

A commercially available Reagent Set[7] was employed in measurement of plasma triglycerides.

A. Reagents

1. (R-1) Alcohol - Ether Reagent 3:1
2. (R-2) Hydroxylamine Hydrochloride Reagent (23 ml H_2O plus 3.5 gm reagent crystals).
3. (R-3) Ferric Chloride Reagent, 10% in N/10 HCL.
4. (R-4) Sodium Hydroxide Reagent, 14%
5. (R-5) Hydrochloric Acid Reagent
6. (R-6) Stock Standard Solution (Tributyrin)
7. (R-7) Working Standard. Dilute the Stock Standard Solution 1 to 20 with Alcohol - Ether Reagent. One ml equals 0.54 mg Triglyceride.

B. Methodology

1. Place 8 ml of Alcohol - Ether Reagent (R-1) in a 10 ml volumetric flask. Add 0.3 ml plasma.
2. Heat to boiling in a boiling water bath with shaking. Allow to cool and make up to 10 ml with Alcohol - Ether Reagent (R-1).
3. Filter through fat-free filter paper[8].
4. Label three cuvettes as follows:

   U - Unknown

   S - Standard

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[7] Laboratory Diagnostics Co. Roselle, New Jersey.

[8] Whatman Filter Paper #43. W.&R. Balston, Limited. London.

B - Blank

5. Place 3.0 ml of filtrate in Unknown cuvette. Place 1.0 ml (R-7) and 2.0 ml (R-1) in Standard Cuvette. Place 3.0 ml (R-1) in Blank cuvette.
6. Add 0.5 ml of Hydroxylamine Hydrochloride Reagent (R-2) to each cuvette and mix.
7. Add 0.5 ml of (R-4) to each cuvette and shake.
8. Stopper all tubes and allow to stand at room temperature for 20 minutes.
9. Add 0.6 ml (R-5) to each cuvette followed by 0.5 ml (R-3). Mix gently until any precipitate formed is dissolved.
10. Read Unknown and Standard in the colorimeter against the Blank at 540 mμ wavelength.

C. Calculations

1. Mg Triglycerides per 100 ml plasma

$$= \frac{\text{OD Unknown}}{\text{OD Standard}} \times 0.54 \times \frac{10}{3} \times \frac{100}{0.3}$$

$$= \frac{\text{OD Unknown}}{\text{OD Standard}} \times 600$$

RESULTS AND DISCUSSION

Data were obtained from experimental and control groups each containing six does. Raw data obtained from body weight measurement, hemoglobin and hematocrit determinations and analysis of selected blood lipids and uric acid values are listed in appendix Tables I through V.

Individual body weights were recorded for all animals on days 1, 7, 14, 21 and 28. Blood was obtained for analysis of lipid, uric acid and hemogram values on days 7, 14, 21 and 28.

Statistical Analysis

An Olivetti Underwood Programma 101, self-contained, desk-top digital computer was programmed for statistical analysis of data. Standard deviation, standard error, coefficient of variation and means were calculated for data obtained sequentially from each animal during the study. Analysis of data obtained from both experimental and control animals grouped by collection dates (Days 1, 7, 14, 21 and 28) was identical. Results of initial statistical analyses are summarized in Table V.

Student's t test was employed to compare differences noted between cholesterol, triglyceride, and uric acid values in experimental and control groups, and the probability value (P) obtained from a standard table. Table VI summarizes statistical analysis of differences noted between groups for each test.

Body Weight

Initial random allocation of animals resulted in placement of

TABLE V

MEAN AND STD. ERROR OF EACH PARAMETER MONITORED FOR EXPERIMENTAL (E) AND CONTROL (C) ANIMALS GROUPED BY DATA COLLECTION DATES

ANIMAL GROUP		PARAMETER									
		Hb[1] (GM%)		Ht[2] (%)		CHOLESTEROL (MG%)		TRIGLYCERIDE (MG%)		URIC ACID (MG%)	
		MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
E	Day 7	12.0	0.4	38.8	1.7	63.3	7.9	202.6	17.7	0.72	0.08
	Day 14	11.8	0.4	37.8	1.0	65.0	4.6	148.8	18.9	0.69	0.07
	Day 21	12.2	0.4	38.0	1.4	64.2	4.7	66.9	11.5	0.61	0.09
	Day 28	12.1	0.4	38.1	1.7	86.7	11.3	102.8	33.8	0.48	0.09
C	Day 7	10.8	0.3	39.0	1.1	77.1	12.5	174.9	33.1	0.69	0.09
	Day 14	12.0	0.0	37.3	0.8	76.9	10.6	233.1	28.1	0.71	0.08
	Day 21	12.9	0.0	38.0	0.6	78.8	10.4	206.6	23.2	0.73	0.14
	Day 28	12.8	0.0	38.5	0.7	79.1	10.1	211.9	32.9	0.78	0.10

[1]Hemoglobin Values
[2]Hematocrit Values

TABLE VI

DIFFERENCES (MG%) BETWEEN INITIAL (DAY 7), DAY 21, AND TERMINAL (DAY 28) CHOLESTEROL, TRIGLYCERIDE AND URIC ACID VALUES OBTAINED FROM EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN, STD. ERROR AND "T" TEST RESULTS

	CHOLESTEROL			TRIGLYCERIDES			URIC ACID		
ANIMAL NO.	7-28	14-28	21-28	7-28	14-28	21-28	7-28	14-28	21-28
E 1	22.3	38.9	25.7	-95.0	42.1	88.6	-0.30	-0.20	-0.11
2	0.0	3.3	0.0	-160.3	-99.6	-14.1	-0.41	-0.37	-0.13
3	8.4	-4.9	1.7	-97.0	-89.4	-12.1	-0.26	-0.26	-0.10
4	28.8	41.0	41.0	-4.3	25.2	149.5	-0.23	-0.21	-0.13
5	76.7	55.5	65.3	-110.0	-62.7	-0.1	0.14	0.18	-0.14
6	5.0	-3.3	1.7	-132.2	-91.5	3.3	-0.42	-0.42	-0.20
MEAN	23.5	21.8	22.6	-99.8	-45.9	35.9	-0.25	-0.21	-0.14
STD. ERROR	11.5	10.8	10.9	21.6	25.8	27.6	0.08	0.08	0.00
C 1	1.8	6.2	12.4	54.3	-10.6	26.8	0.30	0.34	0.21
2	7.4	-3.8	-0.6	83.5	1.8	16.1	0.04	-0.03	0.01
3	4.7	0.0	4.1	29.9	-22.1	-3.7	-0.02	-0.04	0.05
4	9.5	4.8	-3.2	19.7	-73.5	-11.0	0.10	0.13	0.22
5	-10.2	0.5	2.3	23.3	-2.7	45.4	-0.08	-0.13	-0.39
6	-1.5	4.8	-13.5	11.4	-20.5	-41.6	0.18	0.11	0.20
MEAN	1.9	2.1	0.3	37.0	-21.3	5.3	0.09	0.06	0.05
STD. ERROR	2.9	1.6	3.5	11.0	11.1	12.6	0.04	0.06	0.09
"T" TEST	NS	NS	NS	**	NS	NS	**	*	NS

NS Not Significant : *Significant $P < .05$: **Significant $P < .01$

heavier does in the control group. Stratification of animals by weight following randomization was not considered tenable and adjustment to a standard total body weight reference by co-variance analysis was not possible.

The mean increase in weight of the lighter experimental animals (167 gm) was, as expected, larger than that of controls (110 gm) during the experimental period. Larger animals tend to gain weight at a pace much slower than lighter individuals when maintained on an ad libitum feeding regimen for similar periods of time.

The standard error of the mean, of means calculated for body weights of individual animals, was remarkably similar in both experimental and control does (4.0 gm). Therefore, observed differences in weight gain during the experimental period between principal and control animals was attributed not to allopurinol administration but to physiologic differences in growth rates expected to occur between animals differing in initial body weight.

Hemoglobin and Hematocrit Values

All hemoglobin and hematocrit values obtained from both experimental and control animals were within established normal ranges (Hb 8.0 - 15.0 gm% : Ht 25.0 - 45.0 %). Statistically significant differences were not observed, employing the t test at the 5 per cent level, when either individual experimental or control animals or those grouped by collection dates were compared.

Expressed as mean values (of collection date group means) and one standard error of the mean, hemoglobin levels decreased from a control

animal value of 12.5 gm% to 12.0 gm% in allopurinol treated animals with a standard error of 0.0 gm% in both cases. The 0.5 gm% reduction in hemoglobin levels falls far short of statistical significance. However, recent data (91) indicating allopurinol administration may have an adverse effect on hepatic and total body iron storage mechanisms may lend additional importance to this finding. Hemoglobin typing, employing electrophoretic techniques, should be included in subsequent studies on allopurinol to rule out possible production of abnormal hemoglobins in animals under treatment.

Hematocrit values exhibited very little variability in either control or experimental group animals. Values expressed as means of either individual animal tests or from animals grouped by collection dates were almost identical in both animal groups (Appendix Raw Data Table III).

Total Cholesterol and Triglyceride Values

Total cholesterol levels were variable, and no statistically significant differences were observed, employing the t test, between control periods and comparable periods following drug administration when data was obtained from animals grouped by collection dates. However, comparison of mean differences between collection dates showed substantial total cholesterol increases in allopurinol treated animals compared to controls (Fig. III). Therefore, results obtained tend to substantiate the apparent experimentally provoked hypercholesterolemic hyperlipemia in rabbits administered allopurinol previously reported (1). Causal mechanisms are unknown and an assessment of

clinical implications of the hypercholesterolemic response, if verified, will not be possible without further studies.

Although one significant statistical difference was noted between initial and terminal experimental and control animal triglyceride levels marked intragroup variability made meaningful biologic interpretation impossible.

Uric Acid Values

Statistically significant reductions in uric acid levels were expected, and observed, in allopurinol treated animals when compared with controls.

Highly significant ($P < .01$) reductions in uric acid levels were observed when differences between initial and terminal values were analyzed. Significant ($P < .05$) reductions were observed in experimental animals when comparing differences observed between groups monitored on day 14 and 28. Differences between groups during the 21 to 28 day interval approached but failed to reach significance at the five per cent level (Table VI) employing the t test.

Plasma uric acid alterations (reductions) noted in experimental animals were consistent with available data which show that urate levels fall quite slowly after initiation of therapy, plateau, then increase to normouricemic levels only gradually after drug withdrawl because of prolonged oxypurinol half-life (75). Pharmacologic half-life of effective enzyme inhibition is, therefore, dose-dependent on allopurinol administered and rate of oxidation to, and subsequent escape or excretion of, oxypurinol (79).

Return of urate levels in experimental animals to values not statistically different from those of control does would be expected 14 days post-treatment.

SUMMARY AND CONCLUSIONS

Effects of orally administered allopurinol (hydroxyprazolo(3,4-d) pyrimidine) on uric acid, selected blood lipid and hemogram parameters in purebred, Dutch Black and Grey, virgin doe rabbits were investigated. Animals weighing 1435 to 2114 grams were randomly allocated to either an experimental or control group each containing six does. All animals were maintained on a commercially available, pellitized rabbit diet and mineral supplement with feed and water provided ad libitum.

Experimental animals received 50 mg of commercially available allopurinol suspended in one ml of distilled water, orally, daily for 14 days. Control group does received an equal amount of saline for a similar period employing identical techniques (Sham Treatment). Data were obtained and analyzed from both experimental and control animals grouped by collection dates. Individual body weights were recorded for all animals on days 1, 7, 14, 21 and 28. Blood was drawn for analysis of lipid, uric acid and hemogram values on days 7, 14, 21 and 28. Lipid values included packed cell volume and total hemoglobin. Sham treatment of controls and allopurinol administration in experimental animals began on day 7 and terminated day 21.

Statistical analysis included computation of standard deviation, standard error, coefficient of variation and means for data obtained sequentially from each doe and from experimental and control animals grouped by collection dates. The t test was employed to compare

differences noted between cholesterol, triglyceride, and uric acid values in experimental and control groups, and the probability value (P) obtained from a standard table.

A greater mean increase in body weight, during the study period, was expected and observed in initially lighter experimental animals when compared to controls (wt. gain: 167 vs. 110 gm). Observed differences were attributed not to allopurinol administration but to disproportionate growth rates expected to occur between animals differing in initial body weight when they were maintained on an ad libitum feeding regimen for similar time periods.

Statistically significant differences between hemoglobin and hematocrit values were not observed, at the 5 per cent level, when either individual experimental or control does or those animals grouped by collection dates were compared. However, hemoglobin level expressed as a mean value of collection date group means decreased from control values in allopurinol treated animals (Appendix Raw Data Table II). All hemoglobin and hematocrit values obtained were within established normal ranges.

No statistically significant differences in total cholesterol levels were observed between control periods and comparable periods following allopurinol administration when data were obtained from does grouped by collection dates. However, comparison of mean differences between collection dates showed substantial total cholesterol increases in experimental animals (22.6 mg%) compared to controls (1.4 mg%) and tends to substantiate the apparent

hypercholesterolemic hyperlipemia in rabbits administered allopurinol previously reported (1).

Marked intragroup variability made interpretation of triglyceride values untenable. The standard error of sequential triglyceride values (32 mg%) was substantially higher than that of control animals (14 mg%).

Available evidence suggests a causal relationship between allopurinol administration and fluctuations in blood lipid components. However, mechanisms are unknown and an assessment of clinical implications, if any, must await further studies.

Allopurinol was found highly effective in its ability to lower blood uric acid levels. A highly significant reduction ($P < .01$) in urate levels was observed between initial (Day 7) and terminal (Day 28) experimental animal uric acid values when compared to controls. Significant ($P < .05$) reductions were observed when comparing differences noted between groups monitored on day 14 and 28. Differences between groups during the 21 through 28 day interval approached but failed to reach significance at the five per cent level.

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APPENDIX

RAW DATA TABLE I

BODY WEIGHT (GRAMS) OF EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN AND STD. ERROR

ANIMAL NO.	DAY 1	DAY 7	DAY 14	DAY 21	DAY 28
E 1	1757	1932	2030	2086	2044
2	1701	1701	1960	1974	1974
3	1918	1904	1876	1876	1876
4	1435	1505	1547	1533	1477
5	1659	1729	1320	1890	1946
6	1491	1589	1631	1645	1645
MEAN	1660	1726	1810	1834	1827
STD. ERROR	72	68	76	84	89
C 1	1673	1687	1701	1715	1729
2	1890	1988	2016	2044	2002
3	1988	2044	2072	2030	2058
4	2044	2100	2345	2401	2212
5	2030	1988	2030	2016	2030
6	1890	2128	2184	2128	2142
MEAN	1919	1989	2058	2055	2028
STD. ERROR	55	64	86	89	67

RAW DATA TABLE II

HEMOGLOBIN VALUES (GRAMS %) OF
EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN AND STD. ERROR

ANIMAL NO.	DAY 7	DAY 14	DAY 21	DAY 28
E 1	14.4	14.0	13.8	14.1
2	10.4	11.8	11.8	12.0
3	10.8	10.2	10.2	10.2
4	11.8	10.7	11.8	11.9
5	12.4	12.2	13.6	12.2
6	12.2	12.4	12.5	12.3
Mean	12.0	11.8	12.2	12.1
STD. ERROR	0.4	0.4	0.4	0.4
C 1	10.8	12.2	12.0	12.4
2	12.6	11.8	13.6	12.7
3	13.8	11.8	13.8	12.3
4	12.6	12.5	13.8	14.0
5	12.2	12.2	12.4	13.4
6	12.2	11.8	12.2	12.2
Mean	12.3	12.0	12.9	12.8
STD. ERROR	0.3	0.0	0.0	0.0

RAW DATA TABLE III

HEMATOCRIT VALUES (%) OF
EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN AND STD. ERROR

ANIMAL NO.	DAY 7	DAY 14	DAY 21	DAY 28
E 1	44	41	42	43
2	38	36	36	37
3	33	37	32	31
4	35	34	37	36
5	41	40	41	42
6	42	39	40	40
Mean	38.8	37.8	38.0	38.1
STD. ERROR	1.7	1.0	1.4	1.7
C 1	35	36	37	38
2	41	39	39	40
3	42	36	39	38
4	40	40	40	41
5	40	39	38	39
6	36	34	35	35
Mean	39.0	37.3	38.0	38.5
STD. ERROR	1.1	0.8	0.6	0.7

RAW DATA TABLE IV

TOTAL CHOLESTEROL VALUES (MG%)
OF EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN AND STD. ERROR

ANIMAL NO.	DAY 7	DAY 14	DAY 21	DAY 28
E 1	68.5	51.9	65.1	90.8
2	63.5	60.2	63.5	63.5
3	58.5	71.8	65.2	66.9
4	96.5	83.9	83.9	124.9
5	37.3	58.5	48.7	114.0
6	55.2	63.5	58.5	60.2
MEAN	63.3	65.0	64.2	86.7
STD. ERROR	7.9	4.6	4.7	11.3
C 1	65.1	60.7	54.5	66.9
2	66.9	78.1	74.9	74.3
3	59.1	63.8	59.7	63.8
4	62.2	66.9	74.9	71.7
5	139.3	128.6	126.8	129.1
6	70.1	63.8	82.1	68.6
MEAN	77.1	76.9	78.8	79.1
STD. ERROR	12.5	10.6	10.4	10.1

RAW DATA TABLE V

PLASMA TRIGLYCERIDE VALUES (MG%)
OF EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN AND STD. ERROR

ANIMAL NO.	DAY 7	DAY 14	DAY 21	DAY 28
E 1	249.2	112.1	65.6	154.2
2	207.5	146.8	61.3	47.2
3	187.8	180.2	102.9	90.8
4	249.2	219.7	95.4	244.9
5	138.1	90.8	28.2	28.1
6	184.0	143.3	48.5	51.8
MEAN	202.6	148.8	66.9	102.8
STD. ERROR	17.4	18.9	11.5	33.8
C 1	180.0	244.9	207.5	234.3
2	136.2	217.6	203.6	219.7
3	120.6	172.6	154.2	150.5
4	132.6	225.8	163.3	152.3
5	335.4	361.4	313.3	358.7
6	144.6	176.5	197.6	156.0
MEAN	174.9	233.1	206.6	211.9
STD. ERROR	33.1	28.1	23.2	32.9

RAW DATA TABLE VI

URIC ACID VALUES (MG%)
OF EXPERIMENTAL (E) AND CONTROL (C) ANIMALS SHOWING MEAN AND STD. ERROR

ANIMAL NO.	DAY 7	DAY 14	DAY 21	DAY 28
E 1	1.10	1.00	0.91	0.80
2	0.80	0.76	0.52	0.39
3	0.47	0.47	0.31	0.21
4	0.72	0.70	0.62	0.49
5	0.55	0.51	0.83	0.69
6	0.69	0.69	0.47	0.27
MEAN	0.72	0.69	0.61	0.48
STD. ERROR	0.08	0.07	0.09	0.09
C 1	0.90	0.86	0.99	1.20
2	0.65	0.72	0.68	0.69
3	0.49	0.51	0.42	0.47
4	0.71	0.68	0.59	0.81
5	0.99	1.04	1.30	0.91
6	0.39	0.46	0.37	0.57
MEAN	0.69	0.71	0.73	0.78
STD. ERROR	0.09	0.08	0.14	0.10