man have been associated with moderate elevations of serum cholesterol(6). This has usually been explained on the basis of biliary stasis. There appears to be little evidence that chlorpromazine has any striking effect on the usual blood lipid levels found in man. Hollister and Kanter(7), however, report that it may be effective in lowering blood lipid levels of patients with essential hyperlipemia.

Summary. The blood cholesterol levels of cholesterol-fed rabbits given daily intramuscular injections of chlorpromazine fail to rise as high as in rabbits fed cholesterol only. Deposition of lipid in arteries and in the liver is also greatly reduced. Repeated injections of chlorpromazine in rabbits on stock diets may cause transient periods of mild hypercholesterolemia.

1. Katz, L. N., and Stamler, J., *Experimental Atherosclerosis,* Charles C. Thomas, Springfield, Ill., 1953.

2. Dundee, J. W., *Brit. J. Anesthesia,* 1954, v26, 357.

3. Stumpf, H. H., and Wilens, S. L., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 219.

4. Wilens, S. L., and Stumpf, H. H., *Am. J. Path.,* 1955, v31, 275.

5. Gould R. G., *Symposium on Atherosclerosis,* Publication 338, p153, *Nat. Acad. Sci.—Nat. and Res. Council,* Washington, 1954.

6. Moritt, E. R., Meyer, M. A., Snell, A. M., Goldman, M. J., Gibson, J. R., Sullivan, B. H., Webster, J. G., and Stone, R. B., *Gastroenterology,* 1955, v28, 901.

7. Hollister, L. E., and Kanter, B. S., *ibid.,* 1955, v28, 1069.

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Studies with 5-Bromouracil in Rodents and Dogs.* (22684)

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The growth inhibitory activity of 5-bromouracil was first observed in *Lactobacillus casei*(1). Later the substance was shown to be extensively incorporated in lieu of thymine in the deoxyribonucleic acids of bacteria(2, 3,4) and of a T_2 phage of *Escherichia coli*(4). Such findings suggest that the agent may be a thymine antagonist—a supposition supported by the near identity in size of the bromine constituent and of the methyl group of thymine (2). Anti-thymine actions may also be involved in the potentiation by 5-bromouracil of the inhibitory effects of folic acid antagonists in *L. casei*(5). Because of these findings, it has become of interest to ascertain whether 5-bromouracil might potentiate the action of amethopterin in the treatment of human leukemia.† With this in mind the work presented here was initiated as a preclinical study of the pharmacological actions of 5-bromouracil. During the study special interest arose in its catabolic fate *in vivo* because of the presence of crystalline deposits in kidneys or bladders of intoxicated animals. It is the primary purpose of this report to document this finding and to present information concerning the chemical nature of these deposits.

Procedure. Mice, rats and dogs were the subjects of the present investigation. The

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The 5-Bromouracil was purchased from the Dougherty Chemical Co., New York City.

† Personal communication from Dr. J. H. Burchenal.

TABLE I. Toxicity of 5-Bromouracil in Rodents.

Species	Administration	No. of successive daily doses	LD_{50}* (mg/kg/day)	19/20 confidence limits* (mg/kg/day)	S*
Mice	Intraper.	1	1400	1120–1750	1.25
	"	5	700	†	1.13
Rats	Intraper.	1	1700	1420–2040	1.16
	"	5	710	530– 960	1.42
	Oral	5	1410	1040–1900	1.42

* Calculated according to Litchfield and Wilcoxon(11). All animals were observed for 14 days after end of treatment.

† Confidence limits not calculated since mortality for doses used was either >84% or <16% (11).

mice in all cases were male Swiss albino mice (Millerton Research Farms). Male CFW (Carworth Farms Wistar) rats were employed for the toxicological studies while the pathological studies were carried out with the male CFN rats (Carworth Farms Nelson). The latter are a sub-strain of Wistar rat which has been bred free of "chronic murine pneumonia," salmonellosis and bartonellosis (6,7). They tolerate larger doses of most agents which we have recently tested than do CFW rats. Most of the toxicological, hematological and biochemical methods employed have been described (8,9). The 5-bromouracil was administered as suspensions in 0.9% NaCl containing 0.5% CMC (carboxymethylcellulose). The mice and rats received 1 ml of suspension per 100 g of body weight given either intraperitoneally or by stomach tube. The total daily oral dose for each dog was suspended in 50 ml of the saline CMC solution and administered by stomach tube using 50 ml of water to rinse the tube. For intravenous administration in dogs the agent was solubilized in 0.9% NaCl by the addition of molar equivalent amounts of ethanolamine. Because of limitations of solubility it was not practical to give intravenous doses greater than 200 mg/kg/day. Plasma and serum bromide levels were determined by the method of Brodie and Friedman(10). When 5-bromouracil was added to plasma or serum this method did not give rise to the liberation of bromide ion.

Results. Course of intoxication in rodents. Table I presents toxicity studies of 5-bromouracil in mice and rats. The toxicity of this agent was similar in both species. Single lethal doses, 2 g/kg, caused death within 24 hours. Following such doses the animals became weak, dyspneic, and depressed within 2-3 hours and the depression increased progressively until death. Several hours after the injections some animals had red tears and a few showed an orange urine. In animals treated with successive daily doses whether by the intraperitoneal or oral route, depression was noted several hours after each administration of the higher doses (1000 mg/kg/day intraperitoneally or 2000 mg/kg/day by mouth.) Deaths occurred between the second and seventh days in these groups. Ruffled fur was also prominent at 2 to 4 days in the animals given 500 to 1000 mg/kg/day and red-brown lacrymation as well as deep orange urine was observed. Two weeks after the last of these doses the survivors appeared healthy and the experiment was discontinued. At lower doses rodents showed no signs of intoxication. As will be seen below, the administration of 5-bromouracil gives rise to significant concentrations of bromide ion in plasma. This suggested a possible explanation for the depression noted in the treated animals. To test this possibility 2 groups of rats (6 rats/group) were given single, intraperitoneal injections of molar equivalent amounts of bromine as 5-bromouracil and NaBr, 2 g/kg and 1.08 g/kg, respectively. The 5-bromouracil animals died within 24 hours whereas the animals receiving sodium bromide recovered after a period of depression. At one-half of these doses both groups of 6 animals survived with no overt signs of intoxication. These results indicated that the liberation of bromide *in vivo* cannot be solely

TABLE II. Chromatographic and Spectral Findings.

	R_f	Maxima pH 5	Maxima pH 13	Minima pH 5	Minima pH 13
Uracil standard	.25	259	284	228	242
5-bromouracil standard	.37	274	289, 226	247	258
A. Rat kidney deposits	.40	275	290, 226	243	255
B. Rat urine spot 1	.26	259	283	228	242
2	.39	274	289, 225	244	256
C. Dog bladder deposits	.40	275	290, 226	242	256

responsible for the acute deaths caused by 5-bromouracil.

Pathological changes in rats. As mentioned above, pathological changes were studied in CFN rats. For this purpose, 18 rats were given 2 g/kg/day by oral intubation for 4 consecutive days. Six rats, chosen at random, were sacrificed at 96 hours (that is, at 24 hours after the last dose). At this time the average weight loss was 15%. The remaining animals, which had also lost weight, were observed for an additional 2 weeks; at the end of this period they had regained their weight and appeared healthy. In all the sacrificed rats the spleen appeared small. In 2 of the animals, congestion and hemorrhage were noted in the cervical lymph nodes. The kidneys of all were enlarged and, when transected, the cortices appeared edematous. Yellowish-gray crystalline deposits encrusting the papilla were found in the pelvis of each kidney. The bladders were filled with bright red urine. The principal microscopic changes were observed in the urinary tract. In all animals an internal hydronephrosis was present associated with small foci of acute pyelonephritis. Only rare tubular deposits were observed in the papillas. These were pink amorphous masses which only rarely contained needle-like structures. Since many crystalline masses were observed grossly, the paucity of deposits observed microscopically may be accounted for by loss during histological processing. More often rounded bodies which stained dark blue were seen in the medulla. These had the appearance of calcospherites. The basement membranes of all glomerular capillaries were slightly and diffusely thickened. Most of the convoluted tubules contained protein casts. A mild cystitis was present in the bladder associated with edema of the wall; edema was also observed in the prostate. In one animal pink masses similar to those observed in the papilla were present in the lumen of the bladder. In the spleens the red pulp appeared to contain fewer nucleated elements than in control rats. Sections of sternum revealed marked congestion of the marrow. In 5 animals there was a slight decrease of nucleated elements which varied from 10 to 20%. In 1 animal the decrease was 50%. The remaining tissues including lymph nodes, intestines and liver showed no significant changes. The kidney deposits from 2 of the rats mentioned above were removed with a spatula and saturated aqueous solutions were prepared. Descending paper chromatograms were developed with these solutions using Whatman No. 3 MM paper and n-butanol saturated with water. About 20 γ of material was applied to each spot at the origin. After development of the chromatograms, the ultraviolet absorbing components were visualized by means of a 15 watt General Electric Sterilamp equipped with a Corning No. 8963 filter. In this case, as in all chromatographic studies reported herein, uracil and 5-bromouracil‡ standards were used. The ultraviolet absorbing spots were eluted and read against appropriate blanks at pH 5 and pH 13. The results are shown in Table IIA. The R_f as well as the spectrum in water and alkali indicated that for the most part the crystalline material was unchanged 5-bromouracil.

Metabolic fate in rats. Additional studies were made of the fate of 5-bromouracil in rats (CFW). Six animals were placed in individual metabolism cages without food but with free access to 0.9% NaCl in drinking bottles

‡ The 5-bromouracil standard employed was the same as that used in the animal studies. It contained no discernible uracil (Table II).

and were starved for 18 hours prior to and during the experimental period. Three received intraperitoneally 0.5% CMC in saline and the other 3 1 g/kg of 5-bromouracil. The 24-hour urine from each rat was collected and diluted with 2 parts water. Three ml portions were then evaporated to 0.2 ml and the concentrated urines were chromatogrammed as described above. Little or no ultraviolet absorbing material was noted in the control urines. Two distinct spots (No. 1 and No. 2 —Table IIB) were observed on the chromatograms of the urines of rats receiving 1 g/kg. From the R_f of these spots and from their absorption spectra they corresponded to uracil and unchanged 5-bromouracil, respectively. No attempt was made to determine quantitatively the amounts nor ratio of the components present. It should be noted that plasma bromide levels at this time were 26-27 mg/100 ml in the treated animals as compared to 0 in the control rats. Since bromide ion is considered to be distributed throughout the extracellular fluid, that is, in about 25% of body weight, this plasma level would have resulted from the dehalogenation of about 12% of the 5-bromouracil injected. This probably represents the minimum amount of 5-bromouracil which was converted *in vivo* to uracil.

Course of intoxication in dogs. All animals were given a maximum of 10 successive daily doses excepting weekends and were observed for 2 weeks following the last dose. Intravenous doses of 100 and 200 mg/kg/day in 2 respective pairs were well tolerated and the animals showed no untoward signs. Two dogs which received 1 g/kg/day by mouth as well as 1 of 2 dogs which received 0.5 g/kg/day died during or shortly after treatment ended, that is, at 6, 14, and 16 days, respectively. Anorexia was noted at 2 to 6 days prior to death in these 3 dogs. Other signs of intoxication were weight loss and diarrhea. One of a pair of dogs receiving 250 mg/kg/day orally showed a transient anorexia and weight loss but both of this pair survived and were healthy at the termination of the experiment.

To determine the nature of lesions associated with intoxication, 2 dogs were given 1 g/kg/day orally for 4 consecutive days and sacrificed 24 hours after the last dose. At this time biochemical and hematological analyses revealed no significant changes from pretreatment values in the following: glucose, clotting time and bromosulfalein retention in blood; chloride, protein and non-protein nitrogen in plasma; hematocrit, total and differential counts of leucocytes and counts of reticulocytes in blood; and counts of nucleated cells in aspirates of bone marrow from the iliac crest. There was present, however, in the serum of both dogs a high concentration of bromide: 62 mg/100 ml. One of the pair presented a normal picture at autopsy. In the other dog the bladder was found to contain dark red urine which was positive for blood. The platelet count in the blood of this animal had decreased from the initial value of 140 x $10^3/mm^3$ to 7 x $10^3/mm^3$ at time of sacrifice. This dog's bladder was also filled with a dark tan solid mass. Its kidneys appeared normal.

The solid mass was removed from the bladder for further study and the findings are summarized in Table IIC. The chromatographic and spectral studies showed that the only detectable ultraviolet absorbing material present was 5-bromouracil.

In the same animal the only significant lesion observed microscopically was the presence of a few small mucosal hemorrhages in the wall of the bladder. Sternal and vertebral bone marrow, lymphoid tissues and intestine as well as all other tissues were normal. Neither crystals, hydronephrosis nor pyelonephritis were observed in the kidneys. In the other dog no microscopic changes were present in *any* of the tissues.

Discussion. It has been reported that 5-iodo-2-thiouracil is converted to urinary thiouracil in the dog(12) and is de-iodinated in the human(13). While this manuscript was in preparation Barrett and West(14) reported a study of a variety of halogenated pyrimidines in the rat. They found that parenteral administration of 5-bromouracil (40 mg/rat x 1) led to the urinary excretion of uracil which was identified by its behavior in several paper chromatographic systems.

In the present study, it was found that oral administration of 5-bromouracil (1 g/kg x 1)

to the rat gave rise to the excretion of both uracil and 5-bromouracil. The urinary components were studied by spectrophotometric as well as paper chromatographic technics. It was of interest to note a concomitant rise in plasma bromide levels. It was also of interest to note that when 5-bromouracil was given in high repetitive doses to rats and dogs, the foreign deposits which appeared in the kidney of the former and in the bladder of the latter were composed predominantly of unchanged 5-bromouracil. Although crystals were found in only one of the 2 dogs sacrificed, the serum bromide levels in both dogs were equally elevated. Presumably *in vivo* dehalogenation readily occurs in this species. The present investigation has revealed no clear-cut evidence regarding the cause of death in dogs given 5-bromouracil. It may be suggested that crystallization of the agent in the urinary tract and bromide poisoning may both contribute to intoxication. If this agent is to be considered feasible for clinical trial there is a real danger of the development of renal insufficiency or bromism or both as judged from the present findings which were consistent in both rats and dogs.

It should be pointed out that although microbiological evidence indicates that 5-bromouracil interferes with nucleic acid synthesis, lesions were not found in the bone marrow or intestinal epithelium of dogs given toxic doses. Only minimal changes in bone marrow were observed in rats given high doses of 5-bromouracil which were without discernible effect in the intestine of this species. This is in contrast to other agents, such as 6-mercaptopurine(8), which are believed to interfere with nucleic acid synthesis and which have primary sites of action in proliferating tissues such as the bone marrow and the intestine.

Summary. The toxicity of 5-bromouracil has been studied in mice, rats and dogs. Single lethal doses in rodents caused death within 12 to 24 hours. Somewhat smaller amounts when given repeatedly in daily doses gave rise to crystals in the kidney. These have been shown by chromatographic and spectrophotometric methods to be predominantly unchanged 5-bromouracil. The urines of rats given a single high dose of the compound have been found to contain uracil and 5-bromouracil. In dogs deaths are produced by the oral administration of 0.5 to 1.0 g/kg daily excepting weekends for a total of 10 doses. The principal changes noted in fatally intoxicated dogs were weight loss, anorexia, diarrhea and significant concentrations of bromide ion in plasma. In one such animal, the bladder with filled with a deposit of 5-bromouracil.

1. Hitchings, G. H., Falco, E. A., and Sherwood, M. B., *Science,* 1945, v102, 251.

2. Weygand, F., Wacker, A., and Dellweg, H. Z., *Naturforch,* 1952, v7B: 19.

3. Zamenhof, S., and Griboff, G., *Nature,* 1954, v174, 307.

4. Dunn, D. B., and Smith, J. B., *ibid.,* 1954, v174, 305.

5. Elion, G. B., Singer, S., and Hitchings, G. H., *J. Biol. Chem.,* 1954, v208, 477.

6. Cummings, C. N. W., *Carworth Farms Quart. Letter* 1956, no. 40, New City, Rockland County, N. Y.

7. Nelson, J. B., *J. Exp. Med.,* 1951, v94, 377.

8. Clarke, D. A., Philips, F. S., Sternberg, S. S., Stock, C. C., Elion, G. B., and Hitchings, G. H., *Cancer Research,* 1953, v13, 593.

9. Philips, F. S., Sternberg, S. S., Hamilton, L. D., and Clarke, D. A., *Cancer,* in press.

10. Brodie, B. B., and Friedman, M. M., *J. Biol. Chem.,* 1938, v124, 511.

11. Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharm. Exp. Therap.,* 1949, v95, 99.

12. Plummer, A. J., *Fed. Proc.,* 1950, v9, 309.

13. Klein, E. M., *Klin. Wochschr.,* 1953, v31, 17.

14. Barrett, H. W., and West, R. A., *J. Am. Chem. Soc.,* 1956, v78, 1612.

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