vascular damage from a single injection of these drugs, which are normally rapidly metabolized and excreted(14,15), and to the possibility of a localized neurotoxicity of the drugs which indirectly affect the endocrine system(16,17).

Summary. Two amines, serotonin and histamine, when administered either separately or in combination, caused weight increase in prostate glands and histological changes indicative of edema or vascular defect. Pathological changes in testes of treated mice included degenerating seminiferous tubules that contained spermatogonial cells with pyknotic nuclei and giant multinucleate cells. Absence of all spermatogenic cells from tubules was characteristic of advanced degeneration. It is suggested that histological changes in the testes were not necessarily the result of pharmacologic effects of the two amines on vascular structures.

1. Benditt, E. P., Wong, R. L., Arase, M., Roeper, E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 303.

2. Sjoerdsma, A., Waalkes, T. P., Weissbach, H., *Science,* 1957, v125, 1202.

3. Crawford, T. T. B., in *5-Hydroxytryptamine,* ed., G. P. Lewis, Pergamon, New York, 1958, p20.

4. Majno, G., Palade, G. E., *J. Biophysic. and Biochem. Cytol.,* 1961, v11, 571.

5. ———, *ibid.,* 1961, v11, 607.

6. Haddy, F. J., *Circulation,* 1958, v18, 732.

7. Haddy, F. J., Gordon, P., Emanuel, D. A., *Circulation Res.,* 1959, v7, 123.

8. Page, I. H., McCubbin, J. W., *Am. J. Physiol.,* 1956, v184, 265.

9. Toekes, I. M., *J. Pharmacol. Exp. Therap.,* 1957, v119, 354.

10. O'Steen, W. K., *Anat. Rec.,* 1962, v142, 264.

11. Harris, G. W., Jacobsohn, D., Kahlson, G., *Ciba Found. Coll. Endocrinol.,* 1952, v4, 186.

12. Oettle, A. G., Harrison, R. G., *J. Path. Bact.,* 1952, v64, 273.

13. Boccabella, A. V., Salgado, E. D., Alger, E. A., *Endocrinology,* 1962, v71, 827.

14. Goth, A., *Medical Pharmacology,* 1st ed., Mosby, St. Louis, 1961, p134.

15. Udenfriend, S., Weissbach, H., Bogdanski, D. F., *Ann. N. Y. Acad. Sci.,* 1957, v66, 602.

16. Soulairac, A., Soulairac, M. L., *J. Physiologie,* 1957, v49, 381.

17. Soulairac, A., *Annal. d'Endocrinol.,* 1962, v23, 281.

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Metabolism of 9-Ethyl-6-MP-S^{35} and 9-Butyl-6-MP-S^{35} in Humans.* (28307)

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9-ethyl-6-mercaptopurine (9-E-6-MP) and 9-butyl-6-MP (9-B-6-MP) inhibit growth of the 6-mercaptopurine (6-MP) sensitive rodent tumor, adenocarcinoma-755(1). A 6-MP resistant strain of this tumor is cross-resistant to the 9-alkylated derivatives. 9-E-6-MP has also been demonstrated to produce remission when administered orally to chronic granulocytic leukemia patients(2). The 9-alkylated derivatives of 6-MP have been demonstrated to inhibit reproduction of cells in tissue culture(3), but cross-resistance between 6-MP and 9-alkylated derivatives was not observed when the number of carbons in the alkyl group was less than 5.

Evidence has been presented that 6-MP is converted to 6-MP-ribotide by cells sensitive to this drug(4,5,6). The ribotide of 6-MP has been postulated to be the "lethal" inhibitor of 6-MP sensitive neoplastic cells. We have found that the rat is not capable of dealkylating the 9-alkylated derivatives of 6-MP, a prerequisite step to formation of a ribotide from these compounds(7). It would, therefore, appear that free thiopurine might possess carcinostatic activity as has been suggested by Mandel(8). This report deals

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TABLE I. Urinary Excretion of S^{35} by Patients Given 9-Butyl-6-MP-S^{35} or 9-Ethyl-6-MP-S^{35}.*

Patient	Wt (kg)	Mg of S^{35} labeled drug	% of dose S^{35} in 24 hr urine
		9-butyl-6-MP	
1. Normal	50	310	91.4
2. "	50	305	84.5
3. "	18	157	72.0
4. Acute leukemia	16	157	60.0
		9-ethyl-6-MP	
5. Normal	59	200	81.6
6. Chronic granulocytic leukemia	51	200	80.0
7. Polycythemia vera	70	200	70.1

* One μg = 100 - 200 c.p.m.

with further studies of the metabolism of the 9-alkylated derivatives of 6-mercaptopurine by humans.

Methods. 9-E-6-MP-S^{35} and 9-B-6-MP-S^{35} were synthesized by published methods(8). Glucuronidase was obtained from Worthington Biochemical Corp. Spectra were run using a Beckman DU spectrophotometer and enzyme reactions were carried out using the same instrument with attached thermospacers through which 37.5°C water was pumped. Ion-exchange chromatography on Dowex 1-X8 columns was carried out as previously described(7). Assay of S^{35} was performed using a Packard Tri-Carb liquid scintillation counter. The patients were maintained on a metabolic ward and given the labelled drugs orally before breakfast. All urine voidings were collected separately, assayed for radioactivity, frozen and stored at minus 20°C.

Results. From 60 to 91.4% of the radioactivity was found in the urine within 24 hours after administration of labeled drug (Table I). A representative time excretion curve for 9-B-6-MP is shown in Fig. 1. Similar excretion curves for 9-E-6-MP were obtained. Fifty to 70% of the radioactivity was eluted from Dowex 1-X8 columns when the pH was approximately one. The radioactive material in the acid eluent was further purified by chromatographing on Dowex 50-X8, and the spectrum of this purified material is shown in Fig. 2. This compound exhibits a UV absorption maximum at 282 mμ. When it is heated in a boiling water bath for 15 minutes in 0.1 M HCl a compound is liberated with an absorption spectrum similar to that of the original drug. Incubation with glucuronidase at pH 4 and 37.5°C results in liberation of free 9-alkylated drug. The identity of the liberated 9-E-6-MP or 9-B-6-MP was confirmed by rechromatographing the acid or enzyme digest on Dowex 1-X8. The data indicate that the urinary metabolite is the S-glucuronide of the 9-alkylated drug. This compound ac-

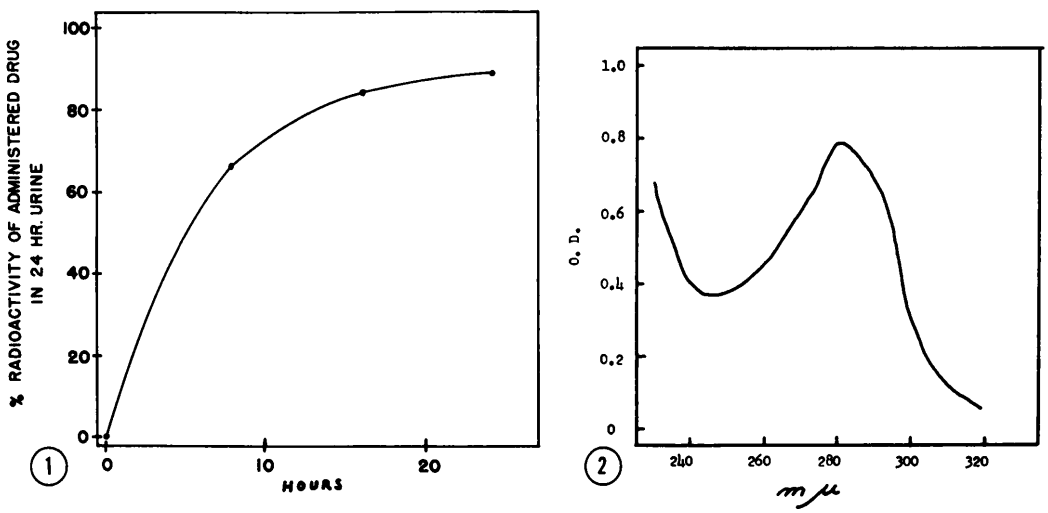

FIG. 1. Percent of radioactivity in urine after orally administered 9-butyl-6-mercaptopurine.
FIG. 2. Spectrum of S-glucuronide-9-butyl-6-mercaptopurine.

counts for 50 to 70% of the radioactivity recovered in the urine. In patients 3, 5, 6 and 7, (Table I) part of the glucuronide was eluted from the Dowex 1-X8 column at pH 8, and in these instances a metal, which is removed by treatment with versene, was chelated with the glucuronide.

The amount of glucuronide present in a given urine sample may be estimated by 1 to 25 dilution of the urine with 0.1 N HCl, heating in a boiling water bath for 15 minutes, and measuring the increase of absorbance at 325 mμ.

The only other metabolite identified in urine of patients given 9-B-6-MP-S^{35} was the unaltered drug which accounted for about 10 to 20% of the total radioactivity. Other radioactive compounds (10 to 20%) have not been identified, but do not appear to have spectra characteristic of thiopurines (310 mμ-360 mμ). The urine of patients 5, 6 and 7, who were given 9-E-6-MP-S^{35}, not only contained glucuronide and unaltered drug but also contained other radioactive metabolites of interest. One of these compounds which was eluted from the column along with uric acid had a spectrum similar to thioxanthine and accounted for about 1% of the radioactivity in the urine. About 15% of the total urinary radioactivity eluted from the column was associated with a compound having a spectrum similar to thiouric acid. Experiments are now being carried out to determine whether this compound and the substance with a spectrum similar to thioxanthine are the 9-alkylated analogs of these compounds.

Discussion. In a previous study(7) we reported that rats given 9-E-6-MP-S^{35} excreted 70% of the drug in the urine within 24 hours. The other labeled catabolites were not identified, but were eluted from Dowex 1-X8 columns with 0.1 N HCl. Most of this unidentified radioactivity has been found to be associated with the S-glucuronide discussed above. We have also isolated a labeled compound with chromatographic properties similar to the glucuronide compound from S-180 ascites tumor cells harvested from mice which were given the radioactive drug intraperitoneally.

From the above, it appears that 70% of the 9-E-6-MP is not de-alkylated and may be recovered as the S-glucuronide in the urine. Fifteen per cent of the remaining radioactivity is associated with a compound which is either 9-ethyl-thiouric acid or thiouric acid. If the compound is thiouric acid this would afford evidence of de-alkylation of the 9-E-6-MP. 9-E-6-MP-C^{14} is now being synthesized from Ethyl-Amine-C^{14} and *in vivo* experiments with this compound should resolve this question. There is no evidence that the butyl derivative is de-alkylated since the urinary radioactivity can be accounted for as the glucuronide and unaltered drug.

Summary. Radioactive 9-B-6-MP-S^{35} and 9-E-6-MP-S^{35} were synthesized and administered to patients. The radioactive metabolites in the urine were separated on Dowex 1-X8 columns and identified by spectrophotometric and chemical analysis. 50 to 70% of the drugs was excreted as the S-glucuronide. About 15% of each drug was excreted in unaltered form. While no other major metabolite was found in urine of patients given the 9-butyl derivative, urine from patients given 9-E-6-MP-S^{35} contained small amounts of radioactive substance having a spectrum similar to thioxanthine, and a larger amount having a spectrum characteristic of thiouric acid. The significance of these observations is discussed.

1. Skipper, H. E., Montgomery, J. A., Thomson, J. R., Schabel, F. M., *Cancer Research,* 1959, v19, 425.

2. Johnson, C. B., Frommeyer, W. B., Hammack, W. J., Butterworth, C. E., Jr., *Cancer Chemotherapy Rep.,* 1962, v20, 137.

3. Dixon, G. J., Schabel, F. M., Skipper, H. E., Dulmadge, E. A., Ducan, B., *Cancer Research,* 1961, v21, 535.

4. Brockman, R. W., *ibid.,* 1960, v20, 643.

5. Brockman, R. W., Debavadi, C. S., Stutts, P., Hutchison, D. J., *Biol. Chem.,* 1961, v236, 1471.

6. Brockman, R. W., Roosa, R. A., Law, L. W., Stutts, P. J., *Cell and Comp. Physiol.,* 1962, v60, 65.

7. Hansen, H. J., Giles, W. G., Nadler, S. B., *Cancer Research,* 1962, v22, 761.

8. Mandel, N. G., *Pharmacol. Rev.,* 1959, v11, 743.

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