ultimately responsible for the antirheumatic activity of salicylate. Therefore, the finding by the method of simultaneous adaptation that gentisate is not oxidized through any pathway known for other aromatic substances, *e.g.*, via catechol, protocatechuate, or homogentisate, introduces the necessity of searching out other likely intermediates, including straight-chain keto-acids. Indeed, if the oxidation of gentisate were quite analogous to that of homogentisate one would expect the production of a β-diketo-, dicarboxylic acid, $C_7H_6O_6$. The qualitative chemical tests carried out so far indicate that such a reaction is entirely possible.

A search through the chemical literature has failed to turn up any helpful information on this score: fumarylpyruvic acid may exist but it has not been synthesized or isolated from natural sources, and must therefore remain for the time being a hypothetical intermediate in the oxidation of gentisate. The problem of the isolation and identification of the oxidation products in the bacterial system must next be undertaken. It may later be possible to apply the findings to a study of gentisate oxidation in man.

Summary. 1. The oxidation by bacteria of salicylate, gentisate, and 2:3-dihydroxybenzoate has been examined with the technic of simultaneous adaptation. 2. Catechol appears to be an intermediate in the oxidation of salicylate. 3. Organisms grown on gentisate and 2:3-dihydroxybenzoate show no adaptation to any likely known intermediates. 4. It is suggested that the oxidation of gentisate may be analogous to that of homogentisate, with formation of a straight-chain β-diketo-dicarboxylic acid.

The authors also wish to thank Drs. K. Meyer and C. Ragan for many helpful suggestions and some of the chemicals, and Dr. J. MacLennon for advice in bacteriology.

1. Turner, J. C., and Lannon, T. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 684.

2. Michotte, L., and Ranaux, R., *Acta Physiother. et Rheumatol. Belg.*, 1952, v7, 142.

3. Stanier, R. Y., *J. Bact.*, 1947, v54, 339.

4. a. ———, *J. Bact.*, 1948, v55, 477; b. cites Tausson, W. O., *Planta* 4, 5, and 7, 1927-29.

5. Happold, F. C., *Biochem. Soc. Symposia*, 1950, No. 5, 85.

6. Evans, W. C., Smith, B. S. W., Linstead, R. P., and Elridge, J. A., *Nature*, 1951, v168, 772.

7. Sleeper, B. P., and Stanier, R. Y., *J. Bact.*, 1950, v59, 117.

8. Kilby, B. A., *Bioch. J.*, 1948, v43, V.

9. Suda, M., Haygaishi, O., and Oda, Y., Osaka, Japan, *Univ. Med. School J.*, 1950-51, v2, 21.

10. Witter, R. F., Snyder, J., and Stotz, E., *J. Biol. Chem.*, 1948, v176, 493.

11. Smith, M. J. H., *J. Pharm. and Pharmacol.*, 1950, v2, 439.

12. Suda, M., and Takeda, Y., Osaka, Japan, *Univ. Med. School J.*, 1950-51, v2, 37.

13. ———, *ibid.*, p. 41.

14. Ravdin, R. G., and Crandall, D. I., *J. Biol. Chem.*, 1951, v189, 137.

15. Fieser and Fieser, *Organic Chemistry*, 1944, D. C. Heath & Co., Boston, Mass., 638.

16. Angelico, F., *Arch. farmacol. sper.*, 1921, v31, 8; cited by Kapp, E. M., and Coburn, A. F., in *J. Biol. Chem.*, 1942, v2, 549.

Received July 28, 1953. P.S.E.B.M., 1953, v84.

Glucosamine and Leukemia.* (20534)

ARTHUR A. MARLOW AND GRANT R. BARTLETT.

From The Scripps Metabolic Clinic, La Jolla, Calif.

Recently Quastel and Cantero(1) reported an inhibition of mouse sarcoma 37 by glucosamine. The rationale of their study was as follows. Hexokinases are able to phosphorylate glucosamine *in vitro*(2,3), the reaction being competitive with glucose. Tumors, presumably being particularly dependent on glycolytic energy sources, might be selectively influenced by a glucosamine block of glucose

* Supported in part by the P. C. Pinson Fund.

TABLE I. Clinical and Hematological Data.

Case:		1*	2†	3	4	5
Age – Sex:		9 – ♀	24 – ♂	28 – ♂	62 – ♀	68 – ♂
Diagnosis:		Acute lymphocytic	Acute histiomonocytic	Chronic myelocytic	Chronic lymphocytic	Chronic lymphocytic
Glucosamine dosage (g/24 hr):		3 days— 1.5 3 " — 3.0 1 " — 4.0 24 " —10.0	1 day— 3.0 3 " — 9.0 2 " —15.0 16 " —20.0	5 days— 6.0 8 " —10.0	1 day— .5 2 " —1.0 1 " —2.0 1 " —3.0 1 " —4.0 3 " —5.0	1 day— 3.0 1 " — 5.0 8 " —10.0
Hb, g/100 ml	Before	11.7	10.5	12.7	13.8	12.4
	After	8.0	11.5 ‡	12.5	11.9	12.2
RBC × 10^6	Before	4.36	3.70	4.49	3.86	4.16
	After	2.85	3.80‡	4.48	3.79	4.26
WBC × 10^3	Before	113	5	29	22	39
	After	298	72	23	23	44
Plats. × 10^3	Before	148	150	98	88	194
	After	80	100	84	96	170
Differential	Before	Blasts. —25.0 Lymphs.—71.0 Neut. — 4.0	Blasts. — 6.0 Monocytes—65.0 Neut. — 4.0 Lymph. —25.0	Blasts.— .5 Lymph.–3.0 Monos.—1.5 Immat. granulocytes—36.0 Mat. granulocytes —59.0	Lymphs.—86.0 Neut. —14.0	Lymphs.—88.0 Monos. — 2.5 Baso. — .5 Neut. — 9.0
	After	No change	No change	No change	No change	No change
Bone marrow	Before	Crowded with blasts	Crowded with blasts—apparent differentiation into monocytes	Permission refused	Mature lymphs.–80.5%	Mature lymphs.—95.1%
	After	Permission refused	No change	" "	No change	No change
Results:		Alive but condition deteriorating 2 mo. after glucosamine was discontinued.	Died 2 days after glucosamine was discontinued. Autopsy.	No change	Died 5 wk after glucosamine was discontinued. Autopsy.	" "

* Courtesy of The San Diego County Hospital, San Diego, Calif., Dr. W. J. Tighe, Chief of Medicine.
† Courtesy of The U. S. Naval Hospital, San Diego, Calif., Capt. A. L. Lawler, M. C., U. S. N., Chief of Medicine.
‡ Multiple transfusions given.

utilization and concomitant diversion of adenosine triphosphate.

Regardless of the mechanism, there was a striking inhibition of the rate of growth of the mouse tumor which was associated with convincing morphological cellular damage and an increase of liver catalase over non-treated controls. In view of these results it was decided to test the effect of glucosamine in human leukemia.

Results and discussion. Glucosamine was purchased from Eastman or prepared from local lobster shells(4). Both preparations were purified by repeated charcoal treatment and crystallization from aqueous alcohol and from water. The resulting snow white crystals gave theoretical reducing values. The compound was administered orally in 0.5 g capsules in divided doses, starting with 1-2 g a day and increasing in 3 or 4 days to as much as 20 g per day. Table I summarizes the hematological findings and the dosage administered.

Except for occasional mild gastric irritation there was no demonstrable toxicity referable to the glucosamine itself. The metabolism of the compound is poorly understood. It has not been found in the urine after large doses in man(5). We were not able to demonstrate it in the urine in significant amount as reducing material, although it possibly was present in conjugated form.

In this limited series of cases there was no favorable influence of the glucosamine on the course of the leukemia.

It should be emphasized that a highly purified glucosamine was employed in this experiment. As a hydrolytic product of the polysaccharide, chitin, it is not unlikely that impure products contain variable amounts of hexosamine polymers. It is also conceivable that such polymers might possess oncolytic activity. Pertinent are the cytotoxic effects of small amounts of bacterial polysaccharides containing glucosamine(6,7). The early observation of glycogen inhibition of rat sarcoma(8) may also be mentioned.

A lack of effect on leukemia does not necessarily indicate that glucosamine will not influence other forms of malignancy.

Summary. Highly purified glucosamine at dose levels as high as 20 g per day for several days did not influence the course of one patient with acute lymphocytic, one with acute histiomonocytic, one with chronic myelocytic and 2 with chronic lymphocytic leukemias.

1. Quastel, J. H., and Cantero, A., *Nature,* 1953, v171, 252.

2. Harpur, R. P., and Quastel, J. H., *Nature,* 1949, v164, 693.

3. Brown, D. H., *Biochim. Biophys. Acta,* 1951, v7, 487.

4. Purchase, E. R., and Braun, C. E., *Organic Synthesis,* ed. by H. Adkins, John Wiley & Sons, Inc., New York, v26, 1946, p. 36.

5. Bergfeld, W., and Kapfhammer, J., *Deut. Z. Verdauungs—u. Stoffwechselkrankh,* 1944, v8, 113-7.

6. Ikawa, M., Koepfli, J. B., Mudd, S. G., and Niemann, C., *J. Nat. Cancer Inst.,* 1952, v13, 157.

7. Shear, M. J., and Turner, F. C., *J. Nat. Cancer Inst.,* 1943-44, v4, 81; Hartwell, J. L., Shear, M. J., and Adams, J. R., Jr., *J. Nat. Cancer Inst.,* 1943-44, v4, 107.

8. Ball, H. A., *Cancer Research,* 1942, v2, 823.

Received July 31, 1953. P.S.E.B.M., 1953, v84.