

that the active form is the free alcohol, and in support of this theory it is significant that the 2 metabolites whose formation *in vivo* has been established are also produced in a peroxidizing fatty acid medium *in vitro*(1).

Summary. Di- α -tocopherone, a recently characterized metabolite of α -tocopherol isolated from animal tissues, was tested for biological activity with respect to resorption-gestation in Vit. E-deficient rats, nutritional muscular dystrophy in rabbits and hereditary muscular dystrophy in mice. The results indicate that this metabolite has less than 5% of the Vit. E activity of α -tocopherol and that it possesses no therapeutic value in treatment of the congenital myopathy. Its probable role in metabolism is that of a terminal oxidation product rather than an active form of Vit. E.

1. Draper, H. H., Csallany, A. S., Shah, S. N., *Biochim. et Biophys. Acta*, 1962, v59, 527.
2. Csallany, A. S., Draper, H. H., *Arch. Biochem. Biophys.*, 1963, v100, 335.
3. Mameesh, M. S., Johnson, B. C., *J. Nutr.*, 1958, v65, 161.
4. Draper, H. H., Goodyear, S., Barbee, K. D., Johnson, B. C., *Brit. J. Nutr.*, 1958, v12, 89.
5. Draper, H. H., Csallany, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1958, v99, 739.
6. Nelan, D. R., Robeson, C. D., *J. Am. Chem. Soc.*, 1962, v84, 2963.
7. Csallany, A. S., Draper, H. H., Shah, S. N., *Arch. Biochem. Biophys.*, 1962, v98, 142.
8. Simon, E. J., Eisengart, A., Sundheim, L., Milhorat, A. T., *J. Biol. Chem.*, 1956, v221, 807.
9. Alaupovic, P., Johnson, B. C., Crider, Q., Bhagavan, H. N., Johnson, B. J., *Am. J. Clin. Nutr.*, 1961, v9, 76.

Received March 18, 1963. P.S.E.B.M., 1963, v113

Lysozyme[†] Levels in the Kidneys of Cancer Patients. (28331)

G. C. PERRI AND MARGRIT FAULK (Introduced by C. C. Stock)

Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York

Lysozyme found to be present in many organs and secretions of humans has been described(1-5). Litwack has isolated chromatographically pure human lysozyme from the kidneys(6) and Jolles and Jolles recently have purified lysozyme from human milk(7). The authors have reported a 70-100 fold increase of lysozyme concentration in the kidneys of Sprague-Dawley rats bearing Jensen Sarcoma (8,9). Surgical removal or spontaneous regression of the tumor results in a return to its normal value in the kidney(10). Similar results have been published for other host-tumor systems by Cappuccino(11). Therefore, it was thought to be of interest to study the lysozyme levels in the kidneys of cancer patients.

Materials and methods. The kidney samples utilized for these experiments were obtained upon autopsy of cancer patients as soon

as possible and kept at -20°C . They were homogenized by the technique already described(7,9). And the resulting extracts, after precipitation of most of the proteins with 1% acetic acid in 95% ethanol and centrifugation, were examined. Lysozyme activity was determined by a modification of Litwack method (12) by addition of supernatant's aliquots to a suspension of acetone dried *Micrococcus lysodeikticus* (Rutgers strain 19) in buffer (chloride-phosphate pH 6.2, OD. 0.600-0.700 $\text{m}\mu$) and measurement of the change of OD. per minute. The results are expressed in mg equivalent of EGG WHITE LYSOZYME* per gram of wet tissue.

Results. The results of analysis performed on 29 kidney samples and reported in Table I show that the lysozyme values obtained vary over a wide range. If the normal value for lysozyme in human kidneys is considered to be 200 γ/g of wet tissue(13), the values found for the cancer-bearing human patient exam-

[†] New name recommended by the Commission on Enzymes of the International Union of Biochemistry: Muramidase (E.C.3.2.1.17).

* Worthington Lot #597.

TABLE I. Lysozyme Content of Kidneys of Cancer-Bearing Human Patients.

No.	Diagnosis	Age	Time of collection PM,* hr	Lysozyme, mg/g
1	Acute leukemia	5	6	.870
2	Embryonal cell ca. of rt. testis	29	3	.468
3	Ca. of rectum	57	11	.193
4	Hodgkin's disease	29	15	.410
5	Ca. rt. breast & lung metast.	57	14	.372
6	Ca. of breast	46	4	.154
7	Acute phase of chr. gran. leukemia	50	20	2.900
8	Ca. of palate	74	6	.748
9	Ca. of ovary (recurrent)	17	6	.016
10	Bronehogenic ca.	38	22	.026
11	Ca. of urin. bladder	53	13	.066
12	Retic. cell sarcoma	77	13	.063
13	Ca. tongue, serum hepat.	77	16	.392
14	Ca. of stomach	53	8½	4.900
15	Ca. of breast	55	21	.056
16	Follicular lymphosarcoma	49	15	.516
17	Ca. of colon	37	14	.115
18	Leukemia	10	6	.102
19	Ca. of lung	62	70	.096
20	Ca. of colon & mult. met.	40	16	.132
21	Rec. ca. of rt. bronchus	61	4	.059
22	Ca. breast & intra. abd. metast.	54	6	.066
23	Ca. stomach & metast. to liver	49	5	.059
24	Leukemia	6	14	.026
25	Ca. of prostate	67	30	.112
26	Polycythemia vera acute leukemia	59	11	.728
27	Ca. of colon	69	7½	.129
28	Synovioma	23	18	.089
29	Ca. of rectum		12	.089

* Post mortem collected.

ined are both greater and lesser than the normal figure and no correlation between the presence of a neoplastic growing mass and levels of lysozyme found in the kidneys is apparent. Some occasional high values are found (cases #7, 22, Table I).

From a pooled sample of the more active extracts crystalline lysozyme was obtained (8) (see Fig. 1), but the amount was too small to permit study of the physicochemical characteristics of the enzyme. It was possible, however, to identify the crystalline material as lysozyme from its lytic activity on *Micrococcus lysodeikticus*, its stability to heat treatment at acid pH's, and its lability above pH 8.0.

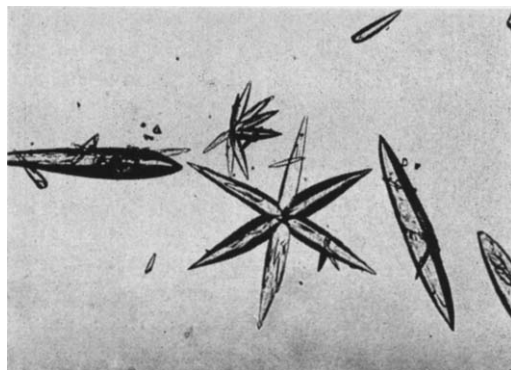


FIG. 1. Crystals of human kidney lysozyme (60 ×).

Work is in progress to isolate more human lysozyme for further study.

Discussion. The results obtained show no corresponding increase of lysozyme in the kidneys of human cancer patients, and are in accord with the results obtained from kidney tissue of experimental animals bearing either spontaneous or chemically induced tumors. On the other hand, *transplantable* tumors are consistently accompanied by an increase of lysozyme levels in the kidneys. These results (7,8,9) fit very well with the view that lysozyme increase in the kidneys (10) of animals bearing such tumors is an indirect response to a challenge of the host defense mechanism either by accompanying bacterial or viral infections or by the tumor tissue itself; spontaneous tumors in experimental animals or in humans do not seem to evoke the same response.

If it is remembered that the lysozyme level in the kidneys of rats bearing Jansen sarcoma usually drops after 18 days of tumor growth, the lack of a consistent increase in lysozyme levels in human kidneys might be attributed to a general impairment of their biochemical functions in the terminal cases examined.

1. Florey, H., *Brit. J. Exp. Path.*, 1930, v11, 251.
2. Ridley, F., *Proc. Roy. Soc. Med.*, (Sec. Ophth.), 1928, v21, 55.
3. Thompson, R., *Arch. Path.*, 1940, v30, 1096.
4. Hiatt, R. B., Engle, C., Flood, C. A., Ramsh, A., *J. Clin. Invest.*, 1952, v32, 721.
5. Prudden, J. F., Lane, N., Levison, J., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 220.
6. Litwack, G., Chakrabarti, S. G., Wojciechowski,

- V., *Arch. Biochem. Biophys.*, 1959, v83, 556.
 7. Jclles, P., Jolles, J., *Nature*, 1962, v192, 1187.
 8. Perri, G. C., Aingstein, R., *Cancer Research*, 1960, v20, 1054.
 9. Perri, G. C., Faulk, M., Mellors, J., Stock, C. C., *Nature*, 1962, v193, 649.
 10. Perri, G. C., Cappuccino, J. G., Faulk, M., Mellors, J., Stock, C. C., *Cancer Res.*, 1963, v23, 431.
 11. Cappuccino, J. G., Reilly, H. C., Winston, S., *Cancer Research*, 1962, v22, 850.
 12. Litwack, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1955, v89, 401.
 13. Oshima, S., Myrvik, R. N., Leake, E. S., *Bact. Proc.*, 1960, p113.
-
- Received March 25, 1963. P.S.E.B.M., 1963, v113