580°C, indicating a possible recrystallization. For this reason, the dry-ashing procedure was carried out at 550°C. However, an apparent partial decomposition of the carbonate may still be noted from the tabulated results.

A preliminary x-ray diffraction powder pattern of this low-temperature extracted bone gives qualitatively the same spacing as bone prepared by other methods. (*9*) Electron-microscope studies of ground extracted bone show no trace of collagen confirming a nitrogen analysis (Kjeldahl) finding of less than 0.1 percent

Bone samples have been prepared by this technique (Figs. 1 and 2) for autoradiography, for demonstration of trabecular stress patterns, for radium analysis of normal bone, and for the separate analyses of tracers in the two fractions of bone. Extracted bone and bone powder have been implanted in dogs and are disposed of in a manner metabolically similar to that of fresh bone and other crystalline material without apparent organic reaction.

References and Notes

1. S. Gabriel, *Z. physiol. Chem.* **18**, 257 (1894).
2. S. Margulis, *J. Biol. Chem.* **93**, 455 (1931).
3. C. D. Crowell, H. C. Hodge, and W. R. Line, *J. Dental Research* **14**, 251 (1934).
4. R. A. Robinson and F. W. Bishop, *Science* **3**, 657 (1950).
5. S. Orell, *J. Bone and Joint Surgery* **35**, 873 (1937).
6. W. F. Neuman and B. J. Mulryan, "Studies on the Surface Chemistry of Bone," AECU-132 (Univ. of Rochester, 1949).
7. Analyses were made by Skinner and Sherman Co., Boston, Mass.
8. Analyses were made by the Soil Mechanics Laboratory, Department of Civil Engineering, M.I.T.
9. R. A. Robinson, *J. Bone and Joint Surgery* **34A**, 389 (1952).

Received March 12, 1954.

Trace Element Content of Cancerous and Noncancerous Human Liver Tissue*

Kenneth B. Olson, George Heggen, Carl F. Edwards, and L. Whittington Gorham

Department of Medicine, Subdivision of Oncology, Albany Medical College and Saratoga Springs Commission Research Laboratory, Saratoga Springs, New York

In a previous publication, a method (*1*) has been described for the quantitative study of 14 trace elements in biological material by spectrochemical means. In experimental rat hepatomas induced by p-dimethylaminoazobenzene (DAB), it has been shown (*2*) that zinc decreased in concentration during the period of liver damage and then increased with regeneration and appeared to be at its peak before gross neoplasia was evident. It seemed of interest to study trace elements in human tissue and the following is a preliminary report on the study of 12 trace elements in the livers of six patients without tumor, two patients dead of carcinoma of the esophagus and with hepatic cirrhosis but without tumor involvement of the liver, four cases with metastasis to the liver from cancer of the gastrointestinal tract, and one case of acute lymphatic leukemia (*3*). Table 1 lists the cases studied. In the six cases without cancer, histological examination of the livers revealed varying degrees of congestion.

* These studies are supported in part by U.S. Public Health Service grant No. C-1121 to the Albany Medical College.

Table 1. Cause of death.

Case No.	Diagnosis	Age
1	Generalized arteriosclerosis; bronchopneumonia	82
2	Myocardial infarction	68
3	Esophagitis and hemorrhage from esophageal varices	76
4	Chronic pyelonephritis	81
8	Cerebral artery thrombosis	80
10	Intestinal obstruction	54
23	Carcinoma of esophagus; portal cirrhosis	59
13	Carcinoma of esophagus; portal cirrhosis	62
17	Carcinoma of rectum*	69
18	Adenocarcinoma of colon*	67
14	Adenocarcinoma of colon*	41
6	Adenocarcinoma of stomach*	39
7	Acute lymphatic leukemia†	9

* Tumor metastasis to liver.
† Hepatic involvement.

Table 2 lists the analyses for zinc and indicates that this element is significantly elevated in the uninvolved portions of livers with metastatic carcinoma. Zinc is increased by 112 percent in the noncancerous portion of the livers with tumor as compared with nontumorous livers. In two patients with carcinoma of the esophagus and with cirrhosis of the liver without metastasis, the zinc concentration was essentially the same as in the noncancerous livers. In these last two cases, the copper concentration was strikingly elevated (165 percent). Zinc has been determined by the method used with an accuracy of ±3.27 percent and copper with an accuracy of ±9.02 percent; thus, it would seem that these findings are of significance. In one specimen, it was difficult to separate all the tumor tissue from liver tissue, and this sample had the lowest concentration of zinc (53.0 ppm), suggesting that considerable tumor tissue was analyzed.

Molybdenum was increased by 37 percent in the liver tissue of patients dying with metastasis to the liver, which is of questionable significance. Manganese, chromium, and tin were present in measurable amounts, but no significant difference was noted in cancerous and noncancerous livers. Nickel, aluminum, silver, lead, and cobalt were present in a few samples, but in most they were below the level of detection for the method of analysis used. Perhaps if larger samples are analyzed or with increased sensitivity of the method, further studies may reveal significant changes in these elements.

In all the tumor tissue studied, the trace elements were markedly decreased as compared with either the liver tissue to which metastasis had occurred or with

Table 2. Zinc content of human livers (parts per million of wet sample).

	Non-cancerous liver	Portal cirrhosis†	Metastatic carcinoma*		
			Uninvolved liver	Tumor tissue	Lymphatic leukemia‡
	36	33	98	26	135
	26	41	68	16	
	43		101	14	
	34		53	18	
	48				
	39				
Avg.	37.7	37.0	80	18	

* Cases No. 17, 18, 14, 6.
† Carcinoma of the esophagus without hepatic involvement; cases No. 23 and 13.
‡ With hepatic infiltration.

noncancerous liver tissue. This is in agreement with other trace-element studies of tumor tissue.

In one case of acute lymphatic leukemia with hepatic infiltration, the liver revealed a marked increase of iron (301 percent), zinc (258 percent), and chromium (233 percent), and cobalt was present in a concentration of 0.96 ppm, whereas the highest concentration in any of the other livers was 0.05 ppm, and cobalt was not detected in most samples. Molybdenum and manganese were reduced, and tin was moderately increased. Lead and silver were present in measurable amounts, but it is not possible to say whether they are significantly increased, since they were found erratically in other livers. Nickel and aluminum were not detected.

Trace elements are probably linked to protein molecules in most instances and are frequently necessary for the activity of enzymes or enzyme systems. Zinc has been shown to be a component of carbonic anhydrase, possibly of uricase, carnosinase, and perhaps some peptidases. Copper is present in tyrosinase. Molybdenum is believed to be a component of xanthine oxidase (*4*). Many other such linkages exist. An attempt will be made to determine whether a trace-element "profile" or pattern exists for host and cancer tissue. It is yet to be determined whether these elements exist in a combined or ionic state. It is also possible that trace-element levels in blood plasma may give a clue to the levels of these elements in the viscera.

To summarize, 12 trace elements have been studied by a spectrographic method in the livers of six persons dying of noncancerous disease, two persons dying of carcinoma of the esophagus and portal cirrhosis of the liver, four persons dying of gastrointestinal cancer with metastasis to the liver, and one case of acute lymphatic leukemia. A significant increase in zinc occurred in the uninvolved portion of the liver in all cases with metastatic malignancy. Copper was elevated in the liver in two cases with portal cirrhosis and no cancer in the liver, although death was due to cancer of the esophagus. Iron, zinc, chromium, and cobalt were significantly elevated in the liver in one case of acute lymphatic leukemia with hepatic involvement.

References and Notes

1. G. E. Heggen and L. W. Strock, *Anal. Chem.* **25**, 859 (1953).
2. Unpublished data.
3. Tissues in this study were obtained from the department of pathology, Albany Medical College, and were frozen and transported to the Saratoga Springs Commission Laboratory for examination. We are indebted to Dr. Arthur W. Wright and his associates for the histological studies and for their cooperation in obtaining material for study.
4. John R. Totter, *et al.*, *Science* **118**, 555 (1953).

Received March 19, 1954.

Heat Death Temperatures and Exposure Times of *Goniobasis livescens*

Carroll B. Nash

Department of Biology, St. Joseph's College, Philadelphia, and Franz Theodore Stone Institute of Hydrobiology, Ohio State University, Put-in-Bay

There are few useful records of heat death temperatures, because many authors neglected the time factor and the older experimenters paid little attention to individual variation. In a study of the heat death of an organism, the proportion of individuals dying out of those exposed should be determined at each of a series of exposure times and temperatures. In addition to the paucity of satisfactory measurements of heat death temperatures and exposure times, the effect of the rate of heating on these characteristics has been generally overlooked.

Dallinger (*1*) and Jollos (*2*) were able to raise the heat death temperature of protozoans by a process of gradual heating, but the heat acclimatization may have been genetical, resulting from selection, since the experiments extended over considerable periods of time. Huntsman and Sparks (*3*) determined the heat death temperatures of marine animals in which the temperature was raised 1°C every 5 min, but did not rapidly heat any individuals for comparison.

The following experiment was conducted to determine the relationship between temperature and exposure time in the production of heat death, and to determine whether there is a differential in the effects of slow and rapid heating on these factors. Individuals of *Goniobasis livescens* were exposed to temperatures up to 41°C for various periods. The snails ranged in weight from 0.3 to 1.1 g, with an average weight of approximately 0.5 g. They were collected in western Lake Erie during the summer and were subjected to experimental heating within a few hours after their collection, at which time the water in their containers had reached a room temperature of 21° to 28°C.

The temperatures of some of the snails were rapidly raised by submerging them in preheated lake water, whereas the temperatures of the others were slowly