

ocular. Between the phototube and the ocular there rotates an opaque disk with a hole at one side, near the periphery. In rotating, the hole in the disk passes over a portion of the ocular field, permitting light to reach the photocell, and thus a portion of the field is scanned repetitively. Pulses registered by the photocell on an oscillograph indicated light transmitted. By proper manipulation, it is possible for an observer to know exactly which areas of observation are being indicated on the oscillograph.

One can substitute for the disk scanning a traveling spot of light from the fluorescent screen of a cathode-ray tube. Different fluorescence emission wavelengths are chosen for specific purposes (in the present experiments, the blue, for the absorption maximum of acriflavine). As shown in Fig. 1, the scanning source is directed at the mirror of the microscope, passes through the object, to the eye of the observer, and to the photocell. The eye sees the entire microscopic field with a single blue line. Pulse height on the oscillograph represents degree of absorption; pulse width represents size of the object interrupting the line.

By these methods, it was possible to study not only fluorescence from the stained smears but also absorption. Also, in living tissue—for example, mesentery—with rapidly moving cells in blood vessels, light absorption of any cells that passed the scanning line could be measured. Photographic methods are available to stop any such rapid measurement at any desired moment.

To make fluorescence studies, an exciting wavelength (4100 or 4350 Å) was used with a yellow filter to eliminate transmitted blue light from the source but to permit yellow fluorescence to reach the "eye." For absorption studies, the yellow filter was removed.

Dye uptake was readily measured by studying increase in pulse height accompanying the gradually increasing amount of stain added to a specimen. With smears from known cancer patients, it was possible, since the pulses indicate not only amount of light but also the area supplying that light, to make simultaneous measurements of nuclear light absorption and nuclear size, a definite aid in picking up malignant cells present.

Readily visible *in vivo* nuclear fluorescence did not provide enough light for adequate oscillograph registration. With faint fluorescence, absorption studies proved satisfactory, the former being proportional to the latter under the conditions used.

Space does not permit more than a word of caution that such quantitation depends upon attention to details of careful absorption studies such as those described by Caspersson (8) and others. The instrumentation described here permits rapid measurement of absorption of light by morphologically identifiable microscopic portions of cells while the cells are alive and moving. The methods provide rapidity of determination, simultaneous measurement of size of object, density, and number of objects (by computers and counters, if desired), at any wavelength. Thus, other wavelengths, other dyes, and other tissues remain to

be explored. The work invites further investigation and will be reported in greater detail.

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Gallium Purification by Single Crystal Growth

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In the course of an investigation of the preparation of intermetallic compounds of the Group III and Group V elements, it became necessary to obtain some high-purity gallium metal. A qualitative spectrographic analysis (1) of the available gallium indicated the presence of 12 elements as impurities (see Table 1, column A). The indicated range (2) of concentration of these impurities was from 0.1 percent down to less than 0.0001 percent. The total impurities indicated were about 0.3 to 0.5 percent.

Consideration of the various chemical means of purification (3, 4, 5) showed them to be complex and time consuming. In addition, many of the chemicals needed in the chemical procedures would have to be purified themselves before they could be used. Physical methods of purification were then considered. Distillation was immediately discarded because of the extreme liquid range and high boiling point of the metal. Recrystallization of the metal seemed to offer the best chance for success. Of the recrystallization methods available, zone purification and single crystal growth by the Kryopoulous technique (6) were considered to be the most promising.

It has been shown by previous work at this Laboratory (7) that the rejection of impurities during single crystal growth is extremely high. Hoffman (3) has shown that gallium grows easily. Therefore, despite the excellent results obtained in the zone purification of germanium, it was decided to try a modification of the Kryopoulous technique for gallium.

Since gallium melts at 29.7° C, a simple water bath held at a constant temperature of 38° C and controlled by means of a thermoregulator, was all that was required to keep the gallium molten. A lucite lid on the water bath held the container of gallium and the thermoregulator in position and prevented excess evaporation from the water bath. A thin-walled glass tube with a pointed tip and funnel top was filled with liquid nitrogen and used as a cold finger. Liquid nitrogen

TABLE 1. Impurities found in gallium.

Impurity	(A) As purchased	(B) After a single growth cycle	(C) After two growth cycles
Mg	TR	VW*	TR
Sn	W	TR-VW	VFTR
Cu	W	VW	TR
Pb	W	VW	TR
Hg	TR	FTR	VFTR
Cd	TR	FTR	
Si	TR	VW*	
Al		FTR*	
Mo	TR		
Ge	TR		
Zn	FTR		
Ca	TR		
Ag	VW		

* See discussion.

Interpretation of symbols: W, 0.1–0.01%; VW, 0.01–0.001%; TR, 0.001–0.0001%; FTR, 0.0001–less than 0.0001%; VFTR, less than 0.0001%; blank means not detected.

was used in preference to some other coolant, such as dry ice-alcohol, because it was easier to maintain the supply of coolant at the tip of the cold finger where there was no solid matter to block the free supply of liquid.

The first container for the gallium was made of Pyrex; but since there seemed to be a pickup of aluminum, magnesium, and silicon (see Table 1, column B) when this was used, a silica container was made and used for the second crop of crystals.

Gallium single crystals were grown with a minimum of effort by the Kyropoulos technique and samples were taken from the tops and tips of all crystals. The first crop of crystals showed a qualitative improvement by a factor of 10 in the concentration of copper, tin, lead, cadmium, and mercury but showed an increase in magnesium, aluminum, and silicon. A recheck on the latter three showed that the impurities were strongly concentrated as a surface film and that in the body of the crystal there had been only a slight pickup of magnesium and no pickup of silicon and aluminum (Table 1, column B). These first crystals were remelted, and a second crop of crystals was grown. Further purification by a second factor of 10 was obtained in the lead, tin, and mercury content and by the elimination of spectrographically detectable amounts of silicon, aluminum, molybdenum, cadmium, zinc, germanium, calcium, and silver, all of which had appeared in the initial qualitative analysis (Table 1, column C).

Thus, after two cycles of crystal growth, there were only five elements remaining that could be detected spectrographically as impurities. Eight others had been reduced so that they were no longer detectable. All the impurities had been reduced by a minimum factor of 10 and some by a factor of more than 1000. The total impurity content of the second crop of crystals

was not more than 0.005 percent and probably nearer 0.001 percent.

Since this purity was sufficient for our purposes, no additional regrowth was attempted. It is felt, however, that by continued growth further purification by at least one factor of 10 could be achieved.

References and Notes

1. The spectrographic work for this investigation was performed by Mr. S. Cress of this laboratory.
2. Throughout this paper, the qualitative spectrographic results have been considered to indicate the approximate range of impurity concentration within the upper and lower limits indicated in the legend for Table 1.
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Estimation of Blood Loss in Hookworm Infestation with Fe⁵⁹: Preliminary Report¹

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A number of workers (1, 2, 3) have expressed the belief that in cases of hookworm infestation the anemia frequently observed could be caused solely by the blood lost from the intestines. In a review of the subject Lane (4) concludes that blood loss is the most probable reason for hookworm anemia. Wells (2) attached *A. caninum* to the intestinal mucosa of living dogs and from iron balance observations calculated the maximum loss of 0.8 ml blood per worm per day, but he allowed for a considerable variation in the blood consumption of individual worms. Hahn and Offutt (5) found a method of studying the blood loss in hookworm infestation of dogs using radioactive iron.

We have used a similar technic on three human patients, who, except for the hookworm ova found in their stools, were otherwise healthy. Our purpose was to determine whether the blood loss per worm per day was constant (improbable) and to what degree the blood loss was responsible for the comparatively low hemoglobin values of our patients.

Our method was briefly as follows. Approximately 40 μ c of Fe⁵⁹, in acid solution containing less than 1 mg of iron, were sterilized, buffered, mixed with 20 ml of the patients' own heparinized plasma, and injected intravenously. Small samples of blood were taken at intervals of 2 or 3 days and the radioactive content measured. After about 8 days, this became approximately constant and the collection of stools was

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