observed on the surface of the cover slip. This material stained positively following the PAS procedure. Preparations examined after longer intervals of exposure to the mantle show, in addition to the features already described, definite fibers which are metachromatic and PAS positive, which is indicative of the presence of polysaccharides. After an interval of 2-3 days, examination of the preparations show that the amebocytes have undergone marked transformations: at first they appear to swell, the transformed cells coalesce, and eventually the mass of cells takes on the appearance of a layer of shell structure.

As a result of the observations referred to above, it is evident that amebocytes participate in and are responsible for repair of the shell. Previous studies have shown that in normal growth of the shell these cells are not extensively involved in this process.

Having established the presence of crystals ( $CaCO_3$ ?) in the amebocytes which become part of the repaired shell structure, we examined the amebocytes under various experimental conditions to ascertain some of the factors which may be responsible for crystal formation.

When the cells are exposed to toluidin blue 1:10,000, the cytoplasm stains metachromatically. In addition numerous vacuoles take up the dye. The fluid within the vacuoles is extremely metachromatic. The vacuole is surrounded by a membrane which contains phosphate. These cells are PAS, positive; alkaline phosphatase could not be demonstrated in the granular amebocytes.

Following the introduction of neutral red to the hemolymph, the dye came to be localized in the vacuoles. At first, the color was indicative of a neutral or slightly alkaline pH. After dessication, the color of the dye changed to red indicating a shift in the pH of the cytoplasm to an acid condition. During the change just described crystals arise in the vacuoles.

Crystals originate in vacuoles within the amebocytes in an environment which is probably in part an acid polysaccharide. When changes occur in the cytoplasm as a result of injury and death of the cell, the buffering apparently breaks down, and calcium is precipitated as crystals of  $CaCO_3$ .

## Screening of Tumors for Nerve Growth Stimulating Properties\*

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Screening experiments which were started during the summer of

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'53 were continued. The following neoplasms were grown as intraembryonic and allantoic grafts in the chick embryo: mouse (E0771, T241L, MA387, C-63); hamster (Hs6, Hs4); rat (Walker carcinosarcoma, Sarcoma 6, Lewis fibrosarcoma, Flexner-Jobling carcinosarcoma); human (Hs#1, H. Ep.#3). Mouse (E0771, T241L, C-63), rat (Lewis fibrosarcoma) and human (H.Ep.#3, Hs#1) tumor grafts grow excellently and can be kept in the laboratory as transmissals on the allantois.

Approximately 1000 transplants have been completed (1953, 1954) from which approximately 400 specimens were obtained. Careful studies on this material have shown that only the mouse sarcomas have the extraordinary properties of stimulating excessive growth in spinal and sympathetic ganglia.

Mouse carcinomas were found to lack this property. Human and hamster tumors were negative. Although rat sarcoma 6 and Walker carcinosarcoma produced mild positive growth responses in spinal and sympathetic centers, the response was not at the same high level as observed with mouse sarcomas.

Experiments were done to explore the effects of nitrogen mustard on the development and maintainance of the induced excessive nerve growth. Chicks with 180 allantoic grafts (implanted at 4 days of incubation) were treated with 0.1 mg. of nitrogen mustard at 11 days of total incubation and sacrificed at 18 days. There was definite evidence of destruction of 180 cells. However, excessive growth in nerve centers (sympathetic and spinal) as well as hyperneurotization of the viscera persisted and was not influenced by the treatment.

## Excretion in the Lobster, Homarus: III

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With the method of Martin Rubin it was found that the gills and carapace are relatively impermeable to Mg; that Mg is absorbed from the stomach; that Mg is concentrated by the nephridia; and that the blood level of Mg is fairly strictly regulated. With lobsters placed in dilute sea water, blood Mg remains near normal levels and the U/P ratio may fall below 1. Normal blood mg averaged 6.8 mM/1. (range: 8.6-5.4) with an average plasma/sea water ratio of 0.13. The average U/P ratio was 1.7 (range: 2.6 - 1.05). The gross Mg cycle is uptake from the gut and excretion by the nephridia.

The average level of blood Ca was 15.6 mM/1. (range: 18.6-13.1). The average U/P ratio was 0.81 (range: 0.53-0.98). The combined values for plasma Mg and Ca were quite uniform averaging 22.4 mM/1. (range: 25.6-20.0). It would appear that Ca is conserved by the nephridia.

By the Fiske-SubbaRow method, normal urine is free from phos-