a 4- and a 5-stage separation. The scheme used in these fractionations is essentially that suggested by Cann and Kirkwood (*3*) for the separation of the components of ovalbumin. A fifth-stage top cut contains the enzyme isolated in the top cell after 5 consecutive refractionations of the collected top cuts. The fifth-stage bottom cut represents 5 similar consecutive refractionations of collected bottom cuts. The enzyme not accounted for in Table 1 is contained in several fractions of intermediate purity. The purest preparation of renal alkaline phosphatase obtained (588 PU/mg TN) has three times the specific activity of the starting material, and appears to be about twice as active as the best preparations in the literature (*4*).

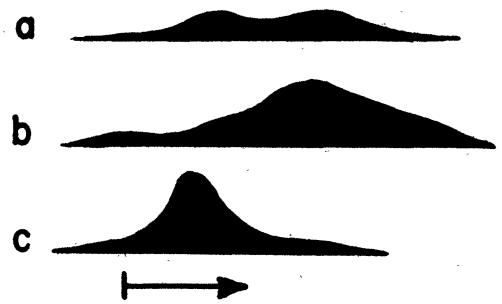

FIG. 1. Electrophoretic patterns (descending boundary). *a*, Expt 2, initial material; *b*, fifth-stage bottom cut; *c*, fifth-stage top cut.

Fig. 1 presents electrophoretic patterns for 3 of the fractions of the second experiment. Pattern *a*, the starting material for the fractionation, exhibits 2 peaks with respective mobilities of 2.2 and 5.0×10^{-5} cm^2/v sec. The broad nature of these peaks indicates the presence of more than 2 components. Pattern *b*, the fifth-stage bottom cut, is complex, but gives indications of components with mobilities of 2.2 (small amount), 4.0, 5.0, and 6.2×10^{-5} cm^2/v sec. The concentration of the more mobile components in this fraction is quite obvious from a comparison of Patterns *a* and *b*. Pattern *c*, the fifth-stage top cut, contains about 50% or more of what appears to be one component with a mobility of 1.7×10^{-5} cm^2/v sec. The presence of slower- and faster-moving components is also indicated. The use of different conditions in the electrophoretic analysis might show this fraction to be more complex than is indicated in the present pattern. However, the enrichment of this fraction in less mobile components is quite clear. The data do not permit conclusions as to the portion of the electrophoretic pattern which represents enzyme, except the obvious inference that it is contained in the slower-moving components of the starting material (*5*); hence no statements as to the absolute purity of the enzyme preparation obtained can be made.

It should be mentioned that the enzyme solutions employed in these fractionations were brown. The pigment, or pigments, responsible moved rapidly in the electrical field and were therefore concentrated quite efficiently in the bottom fractions. Consequently, the use of the Kirkwood apparatus for the removal of certain types of pigments from protein solutions is indicated.

A point of interest is that the present example is perhaps one of the least favorable that could have been chosen, since it was not possible to work at pH values near the isoelectric point of the enzyme. Much greater efficiency should be possible when enzymes that are stable in the region of their isoelectric points are employed.

The following points with respect to the use of electrophoresis-convection in enzyme purification are emphasized: (1) The yields of enzyme are practically quantitative. If the enzyme is sufficiently stable, the only losses that occur are mechanical. (2) The apparatus is much less expensive, and is better adapted for mass separations, than the Tiselius apparatus. (3) Although analytical electrophoresis equipment is of great value in orienting the course of fractionations in the Kirkwood apparatus, it is not essential when materials possessing measurable biological activity are being purified. Specific activity estimations of the fractions obtained are sufficient to guide the purification.

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Effect of Heparin on the Growth of a Transplantable Lymphosarcoma in Mice[1]

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It has been postulated that the gelation that occurs in mitosis prior to the formation of the spindle may have the same physical basis as the mechanism of blood clotting. Heilbrunn and Wilson (*1, 2*) have reported that heparin, in dilute solution, prevents premitotic gelation, thus inhibiting cell division. It was thought of interest to see whether this action of heparin would influence the growth of a rapidly dividing tissue, such as a tumor graft.

Mice of the inbred strain C3H/Jax and the lymphosarcoma 6C3HED were the test materials. The lymphosarcoma grows in 100% of the C3H/Jax mice, forming a discrete mass when transplanted subcutaneously. Fifteen male mice, 2 months old at the start of the experiment and of equivalent weight (approximately 25 g), were divided into three groups of 5 each. Groups 1 and 2 received intravenous injections of

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heparin every 12 hr. Group 1 received 5 USP units, and Group 2, 15 USP units per injection. The first dose of heparin was administered just prior to the subcutaneous inoculation of the tumor. Group 3, the untreated control, was inoculated with tumor at the same time as Groups 1 and 2. Inoculation of the tumor was made by trocar, under aseptic conditions. The course of tumor growth was followed by manual palpation. At the end of the seventh day, there were no differences in size of graft among the three groups. The tumors were large, and the animals debilitated.

Previous determinations of blood-clotting time in C3H/Jax mice showed the average normal time to be 49 sec. This increased to 7.8 min, 30 min after an injection of 5 USP units of heparin. Ten hr after the injection, the time dropped to 2.6 min. Blood samples taken 30 min after an injection of 10 units of heparin failed to clot 4½ hr later. Ten hr after the 10-unit dose, clotting time dropped to 1.8 min.

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Influence of Vitamin D on Deposition of Gallium in Bone of the Rat[1]

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Previous studies in various species have demonstrated that gallium and radiogallium (Ga^{72}), upon parenteral administration as the citrate, are rapidly and preferentially deposited in bone (*1–3*). Clinical studies of the isotope (Ga^{72}) have indicated that it may be useful in diagnostic procedures for the localization of osteogenic and metastatic bone lesions (*4*). To extend our understanding of the behavior of gallium in the normal processes of bone formation and repair, studies were made of the effect of vitamin D upon the deposition of this element in the bones of rats.

Preliminary studies indicated that the oral administration of vitamin D to rachitic rats and chicks, several days before the subcutaneous administration of Ga^{72}, resulted in an increased uptake of gallium by the bones when compared with the rachitic controls, which received no vitamin D.

After these preliminary findings, more detailed studies of rats were carried out. These were designed to show (*a*) effect of age and body weight on gallium uptake in the bones, (*b*) effect of vitamin D on the uptake of gallium in bones of animals raised and maintained on an adequate diet, and (*c*) the effect of vitamin D on deposition of gallium in the bones of rachitic rats.

The test animals were injected subcutaneously with gallium citrate containing 210–240 μc Ga^{72}/mg carrier Ga. The citrate complex (pH 6.5) was prepared as described previously (*5*). The concentration of the Ga^{72} was adjusted so that an injection of 1–2 ml was sufficient to give a dose of 400–600 μc Ga^{72}/kg body weight. The animals were killed 18–24 hr after subcutaneous injection, and the long bones of both hind legs were removed and cleaned free of muscle tissue. The bones were sectioned to separate the shaft from the end portions, and the resulting samples were placed in weighed planchets for radiochemical counting. Gamma counting was done by means of a shielded end window Geiger tube, using a 1750 mg/cm² adsorber to eliminate the β-particles and backscatter. A sufficient number of counts was made (in duplicate) to give a statistical accuracy of at least ±4%. All counts were calculated to the time of injection, allowing for decay time ($t\frac{1}{2} = 14.3$ hr).

A suitable aliquot of a standard sample of Ga^{72} was counted under the same conditions and used to calculate the quantity of Ga^{72} as μc/g dry bone. The initial standardization of this sample was by means of an ionization chamber meter, standardized against a known radium source (*3*).

After completion of counting, the planchets were dried at 110°–120° C for 16 hr, cooled, and weighed. The dried sample was then transferred to a weighed porcelain dish, heated to 1000° C, cooled, and again weighed, and the percentage ash in the dry bone was calculated.

Although efforts were made to standardize the conditions of feeding, sex, age, and weight of the animals of any one series, it must be emphasized that comparison of results of different series must be made with caution. The difference in the dose of Ga^{72}, differences in strain of animals, their age, and diet, are important factors that may contribute to differences between the series. Each series must therefore be considered essentially as a unit.

The series A albino rats were reared and maintained on a commercial diet (Purina Rat Chow), supplemented with fresh vegetables. The immature rats (108 g) were of the same stock as were the young adult animals (237 g).

The series B albino rats were young adults (205 g) of a different stock colony than series A. Littermates were selected at weaning and raised to maturity on an adequate diet. Five of these animals were fed (by stomach tube) 25 USP units of vitamin D daily for 4 days prior to administration of the gallium. The five littermates were given no vitamin D other than that contained in the basal diet.

Series C animals were rachitic rats. These rats were selected from two litters, so that each of the treated and untreated groups contained an equal number of animals from each litter. The young rats were maintained from time of weaning on a rachitogenic diet

[1] The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.