the concept that cells in the middle of the stem liberate substances which are necessary for or contribtue to reconstitution. The constant flow of seawater into the distal end and out of the proximal would prevent any substances liberated into the coelenteron from reaching the distal end. By contrast however, the higher percentage of reconstitution which was recorded for experimental as contrasted with control stems is consistent with expectations if there were inhibitory substances within the coelenteron. The direction of flow would be as successful in preventing inhibitors from reaching the distal end as it was in siphoning off nutritive materials. Since increased acidity of the coelenteron occurs during reconstitution, and builds up to concentrations which are known to be inhibitory when externally applied, the suggestion is made that removal of such substances was responsible for the increased percentages of reconstituted hydranths in our experiments.

*Summary.* An apparatus was constructed which produced a constant flow of filtered seawater through the coelenteron of Tubularia stems. This arrangement was designed to prevent substances liberated into the coelenteron from reaching and influencing reconstitution at the distal end. Reconstitution occurred in spite of removal of such substances and was, in fact, superior to that of control series. Accordingly, it was concluded that although circulating inhibitory substances have been demonstrated, there is no evidence that any substances in the coelenteron influence reconstitution in a favorable direction.

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Received October 20, 1958. P.S.E.B.M., 1959, v100.

## Total Blood, Erythrocyte and Plasma Volumes in Muscular Dystrophic Mice.* (24567)

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The marked decrease in skeletal muscle mass and lowered body weight observed in mice with hereditary muscular dystrophy evoked an inquiry whether or not the circulatory system was in any way altered to adjust to this condition. Since no reports of total blood volume in either mice or man afflicted with muscular dystrophy were found in the literature, experiments to determine total blood, plasma and erythrocyte volumes of muscular dystrophic mice were performed and the results are presented here.

*Methods.* Dystrophic mice were obtained from Roscoe B. Jackson Memorial Laboratory and after a week of acclimatization were studied at 43-52 days of age. Littermate normals of the same sex were used in all except 3 instances in which controls were obtained from other litters but of the same stock and age. Other normal but older mice of the same stock were also used, some of which had become very obese. Plasma volumes were measured by T-1824 dye dilution technic and hematocrit values were obtained by using Van Allen tubes. These procedures were used as previously described(1) except for the dye volume. The plunger of a 0.25 ml hypodermic syringe was calibrated to deliver 0.05 ml of dye through a 26 gauge, ½ inch needle inserted into exposed jugular vein in mice under light ether anesthesia. Dye injection was ac-

* Aided by grant from Muscular Dystrophy Assn. and the Sage and Sackett Research Funds of the Dept. of Zoology, Cornell University.

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TABLE I. Total Blood, Erythrocyte and Plasma Volumes in Normal and Muscular Dystrophic Mice.

| Group & No. | Body wt, g | Total blood vol | Erythrocyte vol | Plasma vol | Hematocrit, % |
|---|---|---|---|---|---|
| | | ml/100 g | | | |
| Normal mice (9) | 27.8 ± 1.1 | 10.9 ± .3 | 5.1 ± .3 | 5.8 ± .2 | 47.5 ± 1.6 |
| Littermates of dystrophic mice (9) | 20.6 ± .6 | 10.9 ± .4 | 5.4 ± .2 | 5.5 ± .3 | 49.2 ± 1.0 |
| Dystrophic mice (9) | 14.6 ± .4 | 11.0 ± .5 | 5.4 ± .3 | 5.6 ± .3 | 49.4 ± 1.2 |
| Obese normal mice (4) | 44.8 ± 2.1 | *8.2 ± .7* | *4.0 ± .4* | *4.2 ± .4* | 48.7 ± .5 |

Means and stand. errors presented; italicized values significantly different, P = 0.01 or less, from appropriate controls.

complished with the aid of binocular dissecting microscope and blood samples were taken by heart puncture. Total blood, erythrocyte and plasma volumes are presented as ml/100 g of mouse body weight. The data were analyzed by the small sample *t* test, and mean differences were considered significant if value of P was 0.01 or less.

*Results.* The results appear in Table I. Two groups (Groups 1 and 2) of normal mice are shown but only those in the second group were of same ages as the dystrophic mice (Group 3). However, mean total blood, erythrocyte and plasma volume body weight ratios and hematocrit values in Groups 1 and 2 are not significantly different.

The dystrophic mice, although much lighter than their normal controls, were identical to them in hematocrit value and had proportionally as much total blood.

The small group of older obese normal mice (Group 4), when compared with Groups 1 and 2 treated as a statistical unit, had significantly less blood/100 g body weight but did have the same distribution of cells and plasma as all the other mice.

In none of the groups was there any evidence of sex differences in the blood picture and the data were thus combined.

*Discussion.* The blood picture obtained in normal mice agrees with published data using similar dye methods(2) and those using radioiodinated albumen(3). In spite of a 33% difference in body weight, due in large part to decrease in muscle mass in the dystrophic mice, the quantities of these blood constituents on basis of body weight were proportionally the same as in controls. Furthermore, it seems unlikely that the vascular space in dystrophic muscles themselves is different from that in controls. Approximately 20% of total plasma volume of mice resides in skeletal muscle which constitutes 40% of body weight (3), and if there were a different ratio of plasma volume to muscle in the dystrophic mouse, it would likely be detected in total plasma volume (and total blood volume assuming a normal hematocrit). Such was not the case.

Certain human muscular dystrophies may involve limited groups of muscles, but in the mouse, the atrophic changes are widespread (4) and vascular development is maintained in constant relationship to body weight. Blood volume determination in muscle biopsy samples was reported for dystrophic and normal muscles of man, and although the results were highly variable, the authors concluded that the blood volumes were not different(5).

Although the effects of anesthesia on blood volume determinations are well known(3), it seems unlikely that anesthesia would nullify any basic difference that might be in the parameters considered in these experiments. The decrease in blood volume in older obese mice can be explained by the well known decrease which occurs with age(2) and also in part by the increase in poorly vascularized adipose tissue.

*Summary.* Plasma volume (dye method) and hematocrit value were measured in normal and muscular dystrophic mice, and from these data total blood and erythrocyte volumes were calculated. These 2 groups of mice possessed almost identical total blood, erythrocyte and plasma volumes per 100 g of body weight, but the muscular dystrophic mice were 33% lighter than their littermate controls.

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Received October 20, 1958. P.S.E.B.M., 1959, v100.

# Localization of 5-Nucleotidase and Non-specific Alkaline Phosphatase By Starch Gel Electrophoresis. (24568)

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Bone, intestine, and possibly liver produce non-specific alkaline phosphatase. A specific alkaline phosphatase, 5-nucleotidase, is present in normal serum(1). It is elevated in sera of patients with biliary cirrhosis and obstructive jaundice of extra hepatic origin, and normal in those with bone diseases(1). The present work describes the electrophoretic separation of serum 5-nucleotidase and non-specific alkaline phosphatase by the starch gel technic. These experiments are part of an integrated study of the biochemical and kinetic properties of these enzymes.

*Methods.* All sera were obtained in the fasting state. The study group included 6 normal adults and 3 normal children, and 20 patients with a variety of disease states, particularly those associated with elevation of serum non-specific alkaline phosphatase. In the latter group were 5 patients with Paget's disease, 2 with osteomalacia secondary to malabsorption, 5 with biliary cirrhosis, 3 with Laennec's cirrhosis, 3 with biliary obstruction secondary to choledocholithiasis and carcinoma, and 2 with sarcoidosis. Non-specific alkaline phosphatase and 5-nucleotidase were determined in sera and starch gel by the method of Dixon and Purdom(1) with the following modifications: 0.3 ml of 0.05 M $MgSO_4$ was substituted for 0.3 ml of water in the pH 9.3 and 7.5 $\beta$-glycerophosphate substrate systems, while 0.3 ml of 0.4 M $MgSO_4$ was used for the pH 7.5 adenosine-5-phosphate substrate system. Serum proteins were separated by zone electrophoresis according to a modification of the method of Smithies(2,3). Idaho potato starch† was hydrolyzed for 70 minutes at 36.4°C and dried to a moisture content of 10.3%. The gel was prepared by heating 13.5 g of this soluble starch with 100 cc of 0.027 M borate buffer, pH 9.05. Electrophoresis was carried out at 12°C at 4.5 volts per cm for 16 hours. The gels were then scored in ½ cm sections and cut in half along the horizontal plane. One half was retained for protein staining while the second half was sectioned in ½ cm blocks and placed directly into tubes containing the respective buffer, substrate, and required Mg concentrations.

*Results.* In sera of both normal subjects and of patients with eleavted non-specific alkaline phosphatase, there appear to be 2 alkaline phosphatases. One of these migrates in the region of alpha-2-globulin and beta-lipoprotein, the other with the beta-globulin components of serum (Fig. 1). Approximately 60% of the alkaline phosphatase migrating with the alpha-2-globulin and beta-lipoprotein is made up of 5-nucleotidase. There is no 5-nucleotidase activity in the alkaline phosphatase that migrates with the beta-globulin fraction (Fig. 1). Total non-specific alkaline phosphatase and 5-nucleotidase recoveries in these starch gel blocks were 90% and 40% of the serum used, respectively. Since borate is a known inhibitor of non-specific alkaline phosphatase(4), we attempted to show the relationship of borate inhibition and its correction by addition of Mg ions. It can be seen from Table I that the inhibition of alka-

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