by magnesium. In control experiments it was found that serial dilution of the homogenate, thereby reducing its phosphatase activity, did not in itself significantly alter the activating effect of magnesium. Comparison of curves II and III demonstrates the distinctly increased sensitivity of intestinal phosphatase to acid inactivation in the presence of magnesium. With kidney homogenates magnesium similarly caused increased sensitivity to inactivation by HCl.

Further observations will be necessary to provide a basis for explaining the manner whereby magnesium increases the sensitivity of alkaline phosphatase to inactivation by HCl. It is pertinent to note, however, that Bodansky (*4*) has found that the activating effects of magnesium and cobalt on purified preparations of rat bone and intestinal phosphatase were markedly altered in the presence of certain amino acids. It is possible that in the present experiments the magnesium-phosphatase complex was more labile and hence more subject to acid denaturation than the noncomplexed form of the enzyme.

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## Growth Effects on Chick Fibroblast Cultures of Fractions of Adult and Embryonic Tissue Extracts Following Differential Centrifugation[1]


R. S. Hoffman, J. A. Dingwall, and W. DeW. Andrus

*Department of Surgery, New York Hospital and Cornell University Medical College, New York City*


In a previous paper from this laboratory (*1*) some of the physicochemical properties of the growth-promoting substances contained in a Tyrode's extract of adult sheep heart have been described. In the present communication, attempts to determine roughly the distribution of the active factors between the macro- and micromolecular fractions of the crude extract, as well as that obtained from chick embryos, are reported. Tennant, Liebow, and Stern (*2*) have previously described high-speed differential centrifugation of embryonic extract.

Preparation of extract of adult sheep heart: Sterile adult sheep hearts denuded of pericardium and epicardial fat were cut up and minced in a blender with 6 times their volume of Tyrode's solution. This mixture was kept in the refrigerator for 24 hr and then centrifuged at 2,500 rpm for 20 min. The decanted supernatant extract could be stored in the refrigerator for several months without losing its activity.

The method of preparation of chick embryonic extract was as follows, being similar to that described by Claude (*3*). Eight-day-old chick embryos were collected under sterile conditions and after washing with Tyrode's solution and freeing of all extraneous material were finely ground with sterile sand and extracted with 6 volumes of Tyrode's solution. The resulting mixture was allowed to stand overnight and then centrifuged for 20 min at 2,500 rpm. The decanted supernatant fluid was used for the experiments.

TABLE 1

SUMMARY SHOWING COMPARISON OF GROWTH EFFECT OF ULTRACENTRIFUGED FRACTIONS OF ADULT AND EMBRYONIC EXTRACTS WITH WHOLE EXTRACTS AND TYRODE'S SOLUTION CONTROLS

| No. cultures | Fluid phase added | Average size of cultures at 7 days ($mm^2$) |
|---|---|---|
| 10 | Adult sheep heart extract, *supernatant* | 68.5 |
| 10 | Adult sheep heart extract, *total* | 56.5 |
| 15 | Adult sheep heart extract, *residue* | 22.0 |
| 5 | Tyrode's solution | 12.0 |
| 10 | Embryonic chick heart extract, *supernatant* | 33.5 |
| 10 | Embryonic chick heart extract, *total* | 31.0 |
| 15 | Embryonic chick heart extract, *residue* | 17.0 |
| 5 | Tyrode's solution | 12.0 |

The extracts of adult and embryonic tissues were each centrifuged in sterile lusteroid tubes in the concentration rotor of an air-driven high-speed centrifuge for periods of 1–3 hr at 30,000 rpm. The resulting closely packed pellets, representing the macromolecular fractions in each case, were resuspended in Tyrode's solution in amounts corresponding to the original volume of extract, and the growth-promoting activity of both the redissolved macromolecular and the supernatant fractions was tested on chick fibroblast cultures growing *in vitro,* by the following technique.

Third passage hanging-drop fibroblast cultures from 7-day-old embryonic chick hearts were divided into halves. Each sister half was placed in a Carrel flask (3.5) containing 0.5 ml chicken plasma, 1 ml Tyrode's solution, and 1 drop of chick embryonic extract in order to facilitate formation of a coagulum. The cultures were allowed to grow in this medium for 48 hr, and the increase of surface area during this period was estimated according to the method of Ebeling (*5*). The 2 fractions of the heart extract were then added to the flasks so that their effects were compared on sister halves of the same cultures, or the activity of each fraction was evaluated by comparison with sister halves of cultures containing only Tyrode's solution in the supernatant phase. Table 1 depicts the results of a series of such experiments.

A survey of the table indicates that the supernatant


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fraction of both embryonic and adult tissue extracts is at least as active as the total before ultracentrifugation. After 6 days the average size of the fibroblast cultures to which the supernatant obtained by ultracentrifugation of adult sheep heart extract had been added was 68.5 mm², whereas that of the sister halves treated with total sheep heart extract was 56.5 mm². The average size of the cultures which received the macromolecular residue redissolved in Tyrode's solution was considerably less, measuring only 22 mm², though it was higher than the average (11 mm²) of the controls to which Tyrode's solution alone was added.

The results obtained with embryonic chick extract were similar, although its stimulating action was of a lower order. The average area of cultures treated with chick embryonic extract was 31 mm², whereas that of the sister halves treated with ultracentrifuged embryonic chick heart supernatant fraction was 33.5 mm². Cultures treated with ultracentrifuged embryonic chick extract residue averaged 17 mm² in area.

Like ourselves, Tennant, Liebow, and Stern (*2*) also found some growth-promoting activity present in the macromolecular fraction of embryonic extract or residue, but these authors make no mention of the activity of the supernatant fraction. Although the slight degree of stimulation obtained with the macromolecular fraction might be due to the presence of additional nutrient material (*4*), our experiments show that the principal growth-promoting properties are retained in the supernatant fraction of the ultracentrifuged extracts.

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## The Influence of Ascorbic Acid Pretreatment on the Leukocyte Response of Rats Exposed to Sudden Stress


**Habeeb Bacchus and Charles A. Toompas**

***Physiology Department, The George Washington University School of Medicine, Washington, D. C.***


It has been observed that ascorbic acid prevents the adrenal hypertrophy typical of animals exposed to cold environments, and enhances the survival of animals exposed to this stress (*1*). It was concluded that the vitamin plays a compensatory role not unlike that of some adrenal cortical hormones. Those findings support the concept of an intimate involvement of ascorbic acid in the functional activity of the adrenal cortex (*2*) and in the utilization of adrenal cortical hormones (*3–6*).

We decided to investigate the nature of this action of ascorbic acid, using the leukocyte picture and adrenal histochemistry as criteria of adrenal cortex activity. Sixteen Wistar female rats (mean body wt, 175 g) were divided into 2 groups: Group I animals were "pretreated" with 150 mg sodium ascorbate (Vitamin C Injectable, "Roche") per rat in 2 intraperitoneal injections; Group II rats received "pretreatment" with saline solution. Total and differential leukocyte counts by Randolph's method (*7*) were made of tail blood of 6 animals in each group. All animals received single subcutaneous injections of epinephrine (0.03 mg/100 g body wt) about 6 hr after pretreatment. Blood counts were taken in the following instances: (*a*) just prior to pretreatment, (*b*) 3 hr after the completion of pretreatment, (*c*) 3 hr after the injection of epinephrine, and (*d*) 24 hr after the epinephrine injection. The remaining 2 animals of each group were killed 1 hr after epinephrine treatment. The adrenals of these animals were treated for the detection of steroids and of ascorbic acid after the methods described previously (*8*).

The pertinent data on the leukocytes are presented in Fig. 1. It is observed that neither pretreatment had any effect on the leukocyte picture. Both groups of animals show lymphopenia ($P < 0.01$) and polymor-

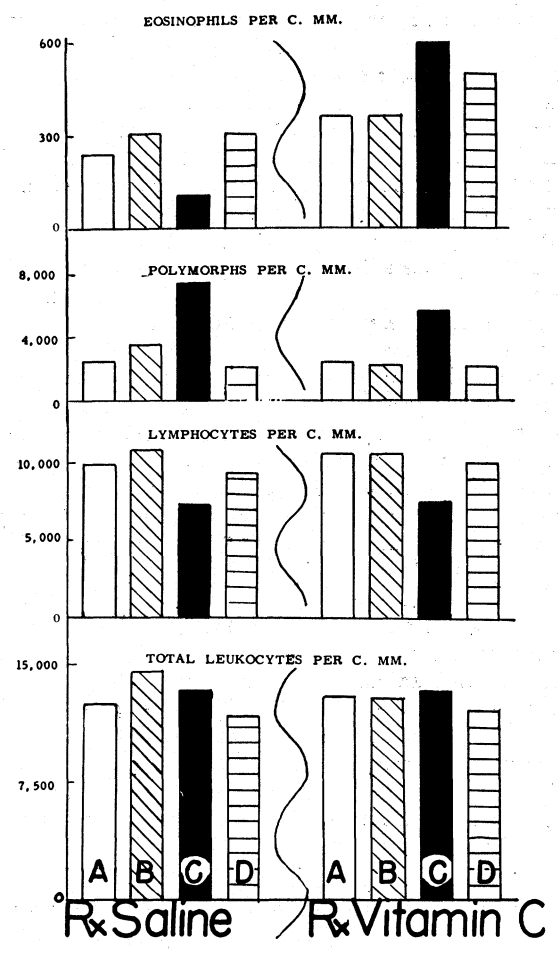


FIG. 1.