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

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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-

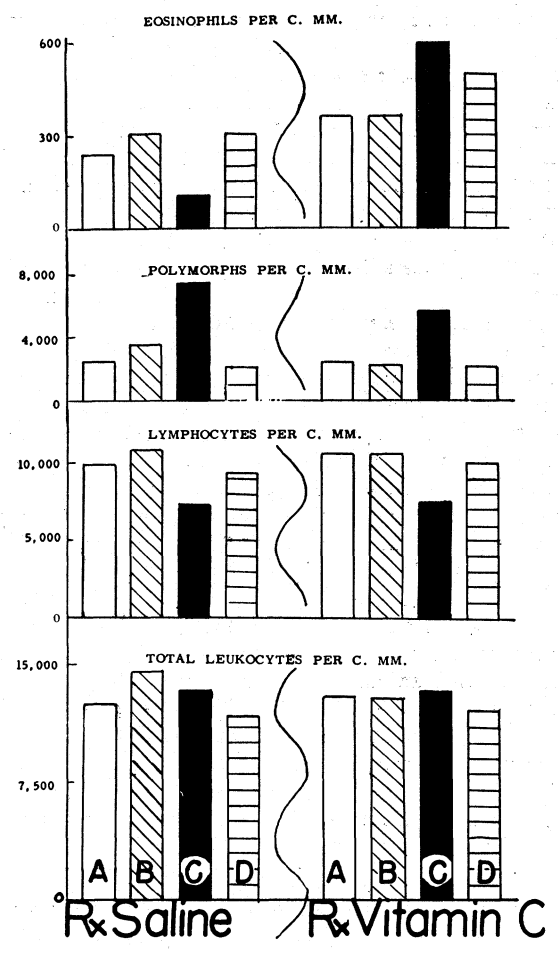

FIG. 1.

phonuclear leukocytosis ($P < 0.01$) 3 hr after the injection of epinephrine. A significant eosinopenia follows the injection of epinephrine in the saline-pretreated group ($P < 0.01$), but there is a significant eosinophilia in the vitamin-pretreated group after the injection ($P < 0.05$). All cellular elements, except the eosinophils of Group II, are back to normal by 24 hr after the epinephrine treatment. A slight eosinophilia still persists in the vitamin treated group.

Histological examination indicated that epinephrine stimulated the adrenals of the saline-treated (Group I) animals. Steroid depletion and sinusoidal depletion of ascorbic acid from the inner zones of the cortex are indicative of the alarm reaction (*9*). The adrenals of Group II animals were completely normal in appearance except for their increased content of ascorbic acid.

The eosinophil and histological tests indicate that ascorbic acid pretreatment prevented signs of the alarm reaction in animals under the stress of epinephrine. It is noteworthy that the lymphocyte picture indicates stimulation of the adrenal cortex in both groups of animals. This is not substantiated by the histological tests. We have concluded that, in this experiment, the eosinophil test of adrenal activity proved to be more accurate than the lymphocyte test. This conclusion is supported by the observation that the lymphocyte response is not under complete regulatory control of the adrenal cortex; Dury (*10*) observed that the lymphopenia of stress does not occur in the splenectomized animal; in the same paper he stated that "the eosinophil therefore seemed most unequivocally, of the leukocytes studied, responsive to adrenal cortical activity alone."

Although the action of ascorbic acid observed in this experiment is very similar in some respects to that of some adrenal cortical hormones, there are certain points of dissimilarity that should be considered. Dugal and Therien observed that the vitamin prevented the hypertrophy of stress but did not cause atrophy of the adrenal; we have observed that pretreatment with the vitamin did not lead to changes caused by adrenal cortical hormones, e.g., lymphopenia, eosinopenia, and polymorphonuclear leukocytosis. In this latter respect the vitamin closely imitates the action of DCA, but it is not known whether this similarity is complete. It is expected that the DCA pretreated animal submitted to stress will not show the lymphopenia which we have observed in the ascorbic acid treated animals. It seems reasonable, therefore, in view of the findings of this experiment to assume a close relationship between the eosinophils and the amount of ascorbic in the organism. Whether this relationship involves transportation of the vitamin or some mass action process is not known. The problem is under further study in our laboratory.

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The Biological Performance of Osmotic Work. A Redox Pump

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There is now much evidence for the view that when yeast in short-period fermentation produces a high degree of acidity the H^+ ions derive from a change such as

$$CtH_2 + 2M^{n+} \rightleftarrows Ct + 2M^{(n-1)} + 2H^+,$$

and the same would appear to hold for the gastric parietal cell, as suggested by Conway and Brady (*1*) and followed by similar views advanced by Crane and Davies (*2*), by Patterson and Stettin (*3*), and by Rehm (*4*).

For yeast it also appears that the immediate process leading to the H^+ ion formation takes place in an outer region which has been identified as the cell wall.

The process occurs cyclically, which means that when the metal catalyst is again oxidized it has either to pass electrons through the inner membrane into the cell or else to pass there physically or to rotate in the membrane; and similarly with the cyclical reduction of Ct. The present communication is not concerned with the exact redox process that may occur, the main object being to indicate a relation between electrical and osmotic energy under such conditions, and the manner in which the relation might be used more generally than in the process of secreting H^+ ions.

Relation between electrical and total energy change when a redox system of type $CtH_2 \leftrightarrows Ct$ *transfers hydrogen atoms to a metal system which retains only the electrons.* It will be assumed that the two systems are in solution in different half cells, with liquid junction:

$$Pt \mid CtH_2,\ Ct,\ HCl \parallel M^+,\ M,\ HCl \mid Pt,$$

and that in each half cell of one-liter capacity there is the same HCl concentration. The platinum electrodes are joined to a source of emf which can be varied so as to allow no current to pass, or to pass very slowly and reversibly.

The electrode reactions are

$$A)\ CtH_2 = Ct + 2H^+ + 2e \quad (1)$$

$$B)\ 2M^+ + 2e = 2M. \quad (2)$$

The electrical work done, when a relatively very small