Ehrlich ascites tumor and Yoshida sarcoma has been obtained from small intestines of mice and rats. 2. Inhibitory properties of the fraction have been demonstrated by pre-inoculation exposure of tumor cell suspensions to the inhibitory material. 3. The intestinal fraction markedly suppresses oxygen consumption of tumor slices, slightly reduces the respiration of normal lymphoid tissues, and has no effect on respiration of other normal tissues tested; an initial respiratory stimulation was observed with ascites tumor, followed by marked depression. 4. The active component of the intestinal fraction appears to be an ethanol and petroleum ether-soluble lipid.

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1. Bennett, Leslie R., Connon, Frank E., and Schoenberg, Melvin D., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 790.

2. Dyer, Helen M., *An Index of Tumor Chemotherapy,* Federal Security Agency, 1949.

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Effect of Stress on Diurnal Fluctuations in Eosinophils of the Laboratory Mouse.* (20213)

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Diurnal fluctuations in the number of circulating eosinophils have been described in several strains of mice(1), and in humans(2,3). Stress, and the adrenal and adrenocorticotrophic hormones cause a decrease in the number of circulating eosinophils in various animals(4,5). The purpose of this paper is to show the effect of sustained cold on the diurnal fluctuations of eosinophils in mice.

Methods and materials. In the first experiment (A) 20 normal male mice of the Jax C-57 Black Strain, obtained from Jackson Memorial Laboratory, were used. The animals were from 6 to 7 weeks old at the beginning of the experiment. They were fed a Rockland Mouse Diet, and food and water were available to the animals at all times. The mice were kept in battery jars which contained a layer of wood chips, 3 to 4 mice being placed in each jar. All of the mice were kept at room temperature for 10 days after arrival to insure full recovery from the rigors of shipment. They were then divided into two groups each containing 10 animals. One group was kept in a cold room at a temperature of 3°C except for the short periods necessary to make eosinophil counts, while the other group, which served as a control, was kept at room temperature. Eosinophil counts were made twice a week for 9 weeks using the direct count method described by Speirs and Meyer (4). Speirs-Levy eosinophil counting slides were used. A day count was made every Sunday between 1:30 and 4:00 P.M. and a night count every Thursday between 6:00 and 9:00 P.M. The interval between counts was thought long enough to permit recovery from any effects on the number of eosinophils resulting from the handling of the animals and the taking of blood. The counts were always made on the control group first and the experimental group second so that the record for each group was standard as to time of day. The actual number of cells per chamber is used as the measure of eosinophil levels of the two groups rather than the number of cells per cubic millimeter of blood since the former shows satisfactorily the relation between the eosinophil levels of the two groups, and for statistical purposes is more conservative. The cells per cu mm of blood may be obtained by multiplying the number of cells per chamber by 10. In a sec-

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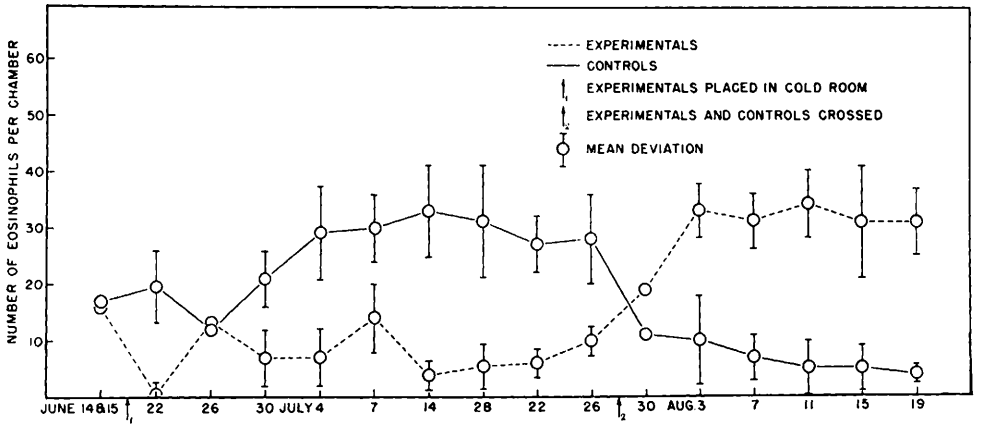

FIG. 1.

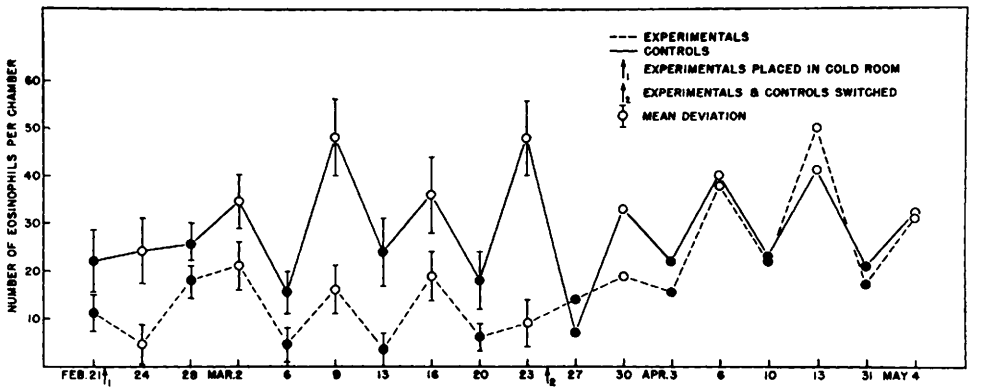

FIG. 2.

ond experiment (B) 2 groups of mice were treated in the same fashion as in experiment A but all of the eosinophil counts were made in the afternoon between 2:00 and 4:00 P.M.

Results. The results of Exp. B appear in Fig. 1 in which there is no suggestion of a marked regular fluctuation in the number of eosinophils. The low point on the graph for the control group which occurred on June 26 is correlated with extremely high temperature and humidity while the high count for the experimental group which occurred on July 9 is correlated with the rise of the temperature in the cold room due to mechanical failure of the cooling mechanism.

A graph showing fluctuations in the average number of eosinophils for the two groups in Exp. A is presented in Fig. 2. The first point on the graph represents a preliminary count made previous to placing the experimental group in the cold. Night counts are represented by dots and day counts by open circles.

After the fifth week the 2 groups in Exp. A were crossed, the experimental group being returned to room temperature and all but two animals of the control group being placed in the cold. The results appear in Fig. 2. All the mice of the control group died shortly after being placed in the cold and no counts were obtained for them. Therefore, the points on the graph for the controls after the fifth week represent the counts for only the 2 control animals not placed in the cold.

From Feb. 21 to Feb. 28 there was no apparent diurnal fluctuation of eosinophils in either group. During this period the mice were still in the process of becoming adjusted to the new situation and it was noted that they were active both during the day and the night. From March 23 to March 30 there was no

TABLE I. Differences between Eosinophil Levels during Day and Night, and between Stressed and Unstressed Mice.

	N*	Diff. ± S.E.†	t	P
I	66	13 ± 5.8	2.9	.02
II	71	10 ± 3.3	3.10	.02
III	78	25 ±11	5.10	.01
IV	64	11 ± 4.1	4.51	.01

* No. of counts.
† Difference between means ± stand. error.
I Difference between night counts of control and exp. groups.
II Difference between night and day counts for exp. group.
III Difference between night and day counts for control group.
IV Difference between night counts for exp. group before and after return to room temp.
P = Probability of a larger value of "t" by chance alone. Probability that a difference as large as that observed would occur by chance alone.

fluctuation of eosinophils among the animals of the experimental group and this is correlated with the period of adjustment immediately following their removal from the cold.

In analyzing the data 4 major categories were considered: the mean difference between night counts, of the 2 groups for the first 5 weeks; the mean difference between the night and day counts within each group from Feb. 28 to Mar. 23; and the mean difference between the counts for the experimental group before and after being returned to room temperature. The preliminary count was not used in any of these calculations. An examination of Table I shows that at the 95% confidence level there is a significant difference for each of the categories tested.

The mice kept in the cold showed a significantly smaller number of circulating eosinophils than did those kept at room temperature. That this reduction can be attributed to the cold is demonstrated by the levels shown by the experimental group after it was returned to room temperature. Examination of Fig. 2 also shows that the diurnal variation persisted in the animals in the cold and was in phase with that of the animals at room temperature. The relative degree of fluctuation is undiminished in the experimental group as may be seen by examination of the graph.

Discussion. It is clear from these results that the depressive effects of cold did not eliminate or even reduce the basic diurnal fluctuation in the number of circulating eosinophils. This suggests that the causal factors underlying the daily rhythm may operate independently of those associated with sustained stress. Though the sustained stress is apparently associated with adrenocortical function no direct evidence for the mechanism of the daily eosinophil rhythm is available. However, it has been found that the number of circulating eosinophils is higher during periods of sleep than during waking activity(2). Halberg and Ulstrom(6) have demonstrated an apparent absence of a 24-hour rhythm in the number of eosinophils in human infants under 15 months of age as evidenced by the absence of an endogenous morning eosinopenia. They suggest that this may be correlated with the absence, in infants, at this age, of the 24-hour periodicity in bodily activity characteristic of adults. They also state that the endogenous morning eosinopenia in mature man is correlated with the initiation of daily activity. Mice are characteristically more active at night than during the day and the difference in the number of circulating eosinophils between these two periods might be explained by this difference in activity. It has been demonstrated that a reduction in the number of circulating eosinophils is correlated with an increase of adrenocortical hormones(4), and that bilateral adrenalectomy eliminates the diurnal fluctuation of eosinophils in mice(7). The diurnal cycle in eosinophils might, therefore, reflect a diurnal variation in the secretion of these hormones brought about by different rates of bodily activity. These observations suggest that nocturnal activity in these experiments acted as a secondary stress superimposed upon the sustained stress of exposure to cold, and that the adrenal gland of the mouse exposed to cold is capable of responding further to the daily stress of nocturnal activity.

Summary. The well known diurnal fluctuation in the number of circulating eosinophils in mice was maintained in animals exposed to continuous cold. It is suggested that a diurnal cycle of activity is responsible for this variation and that the nocturnal activity, acting as a stress, elicits increased activity from

the adrenal cortex which is already responding to the stress of cold.

1. Halberg, F., and Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 846.
2. Tatai, K., and Ogawa, S., *Jap. J. Physiol.*, 1951, v1, 328.
3. Bonner, C. D., *A.M.A.*, 1952, v14, 634.
4. Speirs, R. S., and Meyer, R. K., *Endocrinology*, 1949, v45, 403.
5. Daws, P., and Code, C., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 141.
6. Halberg, F., and Ulstrom, R. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 747.
7. Halberg, F., *J. Lancet*, 1953, v73, 20.

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An Efficient Apparatus for the Redistillation of Water for Biological Purposes. (20214)

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The preparation of water of sufficient purity for critical biological work, and in sufficient quantity, is a perennial problem in biological research laboratories. It is generally agreed that the output of metallic stills such as those of block tin is likely to prove unsatisfactory. Quartz is expensive and the units ordinarily available have a low output and require constant attention. Pyrex glass has proved a satisfactory substitute for most work and there are many types of Pyrex stills available which give excellent results as to quality of product. Most of them, however, are far from automatic and are generally likewise of rather low output. The type of all-Pyrex still described here has been developed after considerable experience. and has been used successfully in preparing nutrients for tissue-culture studies in a number of laboratories. It is relatively inexpensive, and once set up requires a minimum of attention.

The outfit as we use it (Fig. 1) consists of two duplicate units, each made up of 4 items, 3 of which are standard. The units are 1) a standard stove-type heater, usually a 2-burner electric stove with 3-heat burners; 2) two standard flat-bottomed 2-liter distilling flasks (Fig. 2. 3) each with 34/45 ST female joint at the top and side-arm having a 24/40 ST male joint: 3) two standard 22 cm Friedrich condensers. each with a 24/40 ST female connection at the top and standard drip point below, and 4) two constant-level inlet tubes. The inlet tubes* consist of 5 pieces (Fig. 1, 2, 3): first a) there is a feed unit consisting of a 34/45 ST male joint attached above to a 30 mm tubulation and below to one of 20 mm diameter. The lower tubulation is about 200 mm long and when inserted into the flask reaches down to the level at which the water level is to be maintained. The upper tubulation extends upward about 130 mm. Into this is fused concentrically a 10 mm tube 490 mm long so placed as to extend down 440 mm from the point of fusion, that is to about 10 mm from the bottom of the flask, and extending upward 50 mm where it ends in the socket half of an 18/9 ball joint. This forms an inlet for the water supply. From the side of the 30 mm tube a side tubulation 10 mm in diameter is brought out 40 mm, then up parallel to the concentric tube, likewise ending in an 18/9 socket joint. Connecting to this is (b) an "L" shaped 10 mm tube with ball joint at the bottom, a 280 mm vertical arm carrying a 120 mm standard Soxhlet trap, and a 130 mm horizontal arm with socket joint. Parallel to this, and connecting with the concentric tube is (c) a second "L" similar, but lacking the trap. The first "L" connects to a 100 by 100 mm "L" with a single ball joint (d) while the other connects to a 100 by 530 mm "L" with ball joint on the short arm (e). These last are inserted into a No. 12 2-hole rubber stopper. The stopper is

* Built for us by Macalaster Bicknell of Cambridge, Mass.