ALTERATIONS in the number of peripheral blood cells in man and laboratory animals following the administration of ACE (adrenal cortex extract) and ACTH (adrenocorticotropic hormone) have been described in numerous publications and are summarized in recent reviews.1-5 A more limited number of observations has been made on the effects of these preparations on the blood picture of men and animals with lymphatic leukemia. These latter observations have not, however, been species-consistent. In leukemic mice a striking reduction in circulating lymphocytes by ACE has been described,3 whereas similar studies in human chronic lymphatic leukemia indicate a failure to affect the number of circulating lymphocytes with ordinarily potent doses of ACTH and lipo-adrenal extract.4

The purpose of the present report is to describe changes in the peripheral blood picture observed following the administration of purified hog ACTH* and of relatively large doses of aqueous ACE† to persons with infectious mononucleosis and lymphatic leukemia.

MATERIALS AND METHODS

The chief experimental subjects were a group of young adults in good health, a group of young persons with infectious mononucleosis, and several older adults with chronic lymphatic leukemia. At the time of study the white blood cell counts of the leukemic patients ranged from 8,000 to 52,100 per cu. mm. The patients with infectious mononucleosis were studied between the seventh and twenty-fifth day of their disease and all showed significantly high titers of heterophile agglutinin.

When adrenal cortex extract or physiologic saline solution were administered, each was given intravenously in almost all instances at a uniform rate of 5 cc. per minute. No untoward effects were experienced even when 100 cc. of ACE was administered intravenously to a healthy young adult. The single doses were for the most part 25 cc., but occasionally larger doses were used as in the subjects with leukemia, who received 50 cc. either intravenously or intramuscularly. ACTH was prepared by dissolving 25 mg. of the dried powder in 5 cc. of physiologic saline adjusted with NaOH to a final pH of 6.4. The solution was freshly made on the morning of use and 1.5 cc. containing 11.5 mg. were injected into each deltoid muscle. All subjects studied were either in bed or restricted to a sedentary program for the experimental day. Food was limited to a light liquid meal which was not taken until several hours after administration of the test material, and as long before the next succeeding blood count as possible—usually one to two hours. In subjects given ACE or saline intravenously, finger or ear blood for counts was drawn at intervals of 0.5, 1.0, 1.5, 2, 3, and 6 hours after administering the hormone. In subjects given ACTH, counts were made on cutaneous blood drawn 2, 4, 6 and 8 hours after the injection. In most instances, the preliminary blood count was based on three samples drawn at half-hourly intervals prior to the injection; in a few instances, a single preliminary count was done.

Total leukocyte counts were determined by averaging four independent counts from separate pipets of conventional type. Agreement was generally good, although an occasional count was discarded if its

From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

* Furnished by Dr. John R. Mote of the Armour Laboratories, Chicago.
† Furnished by Dr. David Klein of the Wilson Laboratories, Chicago.

732.
value changed the average of the other three by more than 10 per cent. Differential counts at each interval were made on satisfactory Wright-stained cover slip smears, 100 cells on each of four cover slips being counted by two individuals. Direct eosinophil counts were made, using a 0.2 mm. deep Fuchs-Rosenthal chamber and peripheral blood diluted 1:10 with phloxin-propylene glycol. Because of their notorious variability, no values for eosinophils based on differential counts of smears were used in the results reported. The absolute number of each of the other cell types was calculated from the total and differential counts and the value at each interval expressed in terms of its percentage difference from the average preliminary counts.

Observations were made to detect possible changes in the titer of several circulating antibodies following hormone administration. In all subjects with infectious mononucleosis, venous blood was drawn immediately before and four to eight hours after the administration of hormone, for the determination of heterophile agglutinin titers.* In all normal subjects who had had previous recent immunization with typhoid-paratyphoid vaccine (usually in military service), enteric agglutinins against the "O" and "H" typhoid antigens and against paratyphoid A and B were also determined at similar intervals. All enteric agglutinin titers and a number of the heterophile titers were determined at the Connecticut State Laboratory in Hartford, Connecticut by the usual technics.

RESULTS

Results are best reviewed according to the various cell types which were studied.

Lymphocytes. A comparison of figure 1 with figure 2 reveals that an intravenous injection of 25 cc. of ACE produced a variable but definite drop in the number of circulating lymphocytes, becoming evident at one hour after the injection and persisting for two hours. The response was not the same in all individuals. In patients with infectious mononucleosis the rapidity and magnitude of the fall in lymphocytes were in the same range as those of the group of normal subjects. It may be noted that the normal subjects receiving ACE and saline were the same individuals, given each injection on different days of study.

Two of the 13 subjects in figure 1 deserve special mention. One, a normal male, received an injection of 100 cc. of ACE intravenously over a twenty-minute period without any subjective or objective ill effects. The resulting fall in lymphocytes was the greatest obtained in the group of normal subjects. The second, a 27 year old woman with Addison's disease, after 25 cc. of ACE showed a reduction in lymphocytes which was greater than in any of the normal subjects studied. Both of these subjects were included in the control groups receiving saline injections. The first received 100 cc. of 0.9 per cent saline solution containing 1 part per million of epinephrine.† This produced a definite reduction in lymphocytes but less than that which followed the administration of ACE to the same subject. The patient with Addison's disease responded to 25 cc. of 0.9 per cent saline in a manner indistinguishable from that of the normal subjects.

Four patients with chronic lymphatic leukemia were given ACE. One received 50 cc. intramuscularly (in six sites) and the other 3 were given 50 cc. intravenously. The effect of these injections on the lymphocyte count was negligible, as shown in figure 3, although the dose used was twice as great as that which was effective in the subjects of figure 1.

* Dr. Alfred S. Evans of the Department of Preventive Medicine, Yale University School of Medicine, kindly determined a number of heterophile agglutinin titers.
† Dr. Klein, of Wilson Laboratories, states that Wilson's Adrenal Cortex Extract contains less than 1 part of a million of epinephrine by bioassay.
ACE AND ACTH, AND CHANGES IN CIRCULATING LEUKOCYTES

Fig. 1.—Per cent of change in number of circulating lymphocytes in 13 subjects after intravenous ACE. Seven normal subjects (black circles); 5 with infectious mononucleosis (open circles); 1 with Addison's disease (triangles).

Fig. 2.—Per cent of change in number of circulating lymphocytes in 9 subjects after intravenous NaCl. Eight normal subjects (black circles); 1 with Addison's disease (triangles).

Following intramuscular injections of ACTH, a small drop occurred in the 3 normal subjects and in 5 patients with infectious mononucleosis. An attempt was made to compare the effect of ACTH and ACE in the same individuals by giving both preparations to patients with infectious mononucleosis on different days.
RICHARD H. SAUNDERS AND ELIJAH ADAMS

Fig. 3.—Effect on circulating lymphocytes of 4 patients with chronic lymphatic leukemia of 50 ml. of ACE. Three patients injected intravenously (open circles); 1 injected intramuscularly (black circles).

Fig. 4.—Per cent of change in number of circulating lymphocytes after intramuscular injection of ACTH in 11 subjects. Three normal subjects (black circles); 5 with infectious mononucleosis (open circles); 3 with lymphatic leukemia (triangles).

The effect can be seen by comparing the points for these subjects in figures 1 and 4. The data are too few for detailed analysis but in general the drop in lymphocytes occasioned by both preparations seemed similar for the first three hours after the injection.
ACE AND ACTH, AND CHANGES IN CIRCULATING LEUKOCYTES

Fig. 5.—Per cent of change in number of circulating eosinophils after intramuscular injection of ACTH in 10 subjects. Three normal subjects (black circles); 4 with infectious mononucleosis (open circles); 3 with lymphatic leukemia (triangles).

Fig. 6.—Per cent of change in number of circulating eosinophils in 7 subjects after intravenous ACE. Five normal subjects (black circles); 1 with infectious mononucleosis (open circles).

Three patients with lymphatic leukemia showed essentially no change in number of circulating lymphocytes after ACTH. It is interesting to note, however (fig. 4), that the circulating lymphocytes in these patients tended to rise after the fourth
Fig. 7.—Per cent of change in number of circulating eosinophils in 5 normal subjects after intravenous NaCl.

Fig. 8.—Per cent of change in number of circulating polymorphonuclears in 14 subjects after ACE. Eight normal subjects (black circles); 3 with infectious mononucleosis (open circles); 1 with Addison's disease (triangles).

hour, in a manner similar to that observed in the other subjects receiving this preparation.
**Eosinophils.** Following ACE, a fall in eosinophils was obtained in 5 of 7 subjects on whom direct counts were made (fig. 6). That saline produced no consistent effect on eosinophils is illustrated by the random scatter of the points of figure 7.

The effect of ACTH on eosinophils is shown in figure 5. All 10 subjects, including the patients with leukemia, responded in a manner comparable to that obtained for normal individuals by other observers. Although the magnitude of the response to a single small injection, such as this, covered a wide range, there is no apparent difference in the response of the three types of subjects.

**Polymorphonuclear Leukocytes.** Under the conditions of our study, a rise in polymorphonuclears (exclusive of eosinophils) occurred after both ACE and ACTH. From figure 8, however, one can see that the rise after ACE did not occur until the second hour after the injection. Since the lymphocyte effect was noted fully an hour earlier, the rise in polymorphonuclears could hardly be the cause of the lymphocyte drop. In the case of ACTH there was an average elevation of polymorphonuclears at the first observation period, two hours postinjection. This persisted virtually unchanged through the eight hours of the study, while the lymphocytes first dropped and then rose after the four-hour period; hence, there again seemed to be little inverse relationship between the response of the two types of cells.

It can be seen from figures 8 and 9 that the rise of polymorphonuclears after...
ACTH is greater, more consistent and more prolonged than that which followed an injection of ACE. Individual variation, however, was great in each group.

Monocytes. The percentage of monocytes present in any series of smears was so variable that interpretation was impossible.

Antibody Titters. Six subjects receiving ACE and 7 receiving ACTH were studied for possible changes in the level of circulating antibodies after the injections. In all of these subjects significant antibody levels of either heterophile or enteric agglutinins were present before the injections were given. In none of them was there a change in antibody titer greater than one tube dilution, in the postinjection serum samples. Such small changes, in the few instances in which they occurred, were considered to be within the limits of accuracy of the technics employed.

Discussion

The present data are consistent with previous observations of failure to demonstrate a drop in lymphocytes in persons with chronic lymphatic leukemia given ACTH. We also noted the previously reported observation that the eosinophil response is preserved and that the failure of the lymphopenic response is probably not an adrenal failure since ACE is as ineffective as ACTH. Changes in the neutrophils in these patients cannot be evaluated because of the scarcity of this cell in stained smears.

The interpretation of this deviation from the normal response can be no clearer than the mechanism of the latter itself. One should find it difficult, however, to embrace the notion that this represents an essential difference between mouse and human lymphatic leukemia in view of the great dosage differences used in testing the two species. One possible explanation requiring consideration is that in terms of absolute number, the quantity of cells affected by a given amount of hormone, either exogenous or endogenous, may be relatively constant. An apparently insignificant change in the number of circulating lymphocytes in a subject with chronic lymphatic leukemia, may actually involve a number of cells great enough to represent a considerable percentage reduction in the presence of a normal leukocyte count. More information on this question might come from studies of the effect of ACTH and ACE in some of the benign lymphocytoses.

Whatever factors determine the distinctiveness of subjects with lymphatic leukemia in their hematologic responses to adrenal cortical stimulation or cortical hormones, it would seem that blood changes in patients with infectious mononucleosis are indistinguishable from those in normal subjects in every respect after both ACE and ACTH. It is worth noting, however, that although in this group initial lymphocyte counts were considerably elevated, the percentage changes in all cellular constituents after hormone injection were essentially similar to those observed in the normal subjects, suggesting that the explanation offered above for the failure of ACE to produce a detectable drop in lymphocytes in chronic lymphatic leukemia is not entirely sufficient. In view of current interest in endocrine influences on blood cells there have been surprisingly few published accounts of changes in the blood picture of human subjects following administration of the ordinary adrenal cortex extracts available for therapeutic use, the bulk of hemato-
logic observations in humans having been made with ACTH. The present observations justify the conclusion that a significant drop in the absolute number of lymphocytes can be expected after the administration of as little as 2.5 cc. of aqueous ACE by vein—this dose being characterized as small by comparison with the doses per unit of body weight previously used in similar studies of the blood cell responses in laboratory animals. The changes in the number of lymphocytes are not sufficiently consistent to appear in every individual examined, however, nor are they of sufficient frequency to be evident except in a group of observations.

In the single subject given a large dose of ACE by vein, it is of interest that the lymphocyte drop was in the same range (although among the most marked of the individual responses) seen with several persons given only 25 cc. of ACE. That the reduction in lymphocytes in this instance cannot be unequivocally interpreted as resulting from corticosteroid administration is suggested by the control observations when the same individual was given 100 cc. of physiologic saline containing 0.1 mg. of epinephrine. The difference in eosinophil response under these respective conditions and the fact that a definite salt retention occurred in this subject after administration of ACE but not after epinephrine-containing saline indicates that this quantity of epinephrine did not stimulate endogenous ACTH secretion to a significant extent. As already noted, 0.1 mg. of epinephrine is probably in excess of the quantity contained in 100 cc. of ACE. In any case, all other observations were made after giving amounts of one half to one-quarter this volume of ACE. It would appear, however, that any studies of the effect of aqueous ACE in humans—particularly when doses per unit of body weight approaching the amounts given to animals are employed—must consider the possible effects of epinephrine contained in the extract.

Observations of the comparative effect of ACE and saline in the single subject with Addison's disease studied, suggest the possibility that absence of adrenal function may exaggerate the biologic effects of exogenous adrenal hormones in respect to hematologic changes, as well as in fluid and electrolyte changes, in which exaggerated responses in the addisonian individual are well known. Dougherty and White reported hematologic changes of almost identical magnitude in groups of intact and adrenalectomized mice given 0.5 ml. of ACE respectively but differences in response between such groups of animals might well be masked by such relatively large doses.

**Summary**

Reduction in absolute numbers of circulating lymphocytes and eosinophils was observed after administering 25 cc. intravenous doses of aqueous ACE to normal adults. Such changes did not occur after a comparable intravenous dose of physiologic saline. Patients with infectious mononucleosis showed hematologic responses to ACE and ACTH both qualitatively and quantitatively similar to those observed in normal persons, while a small group of persons with chronic lymphatic leukemia failed to respond with a characteristic fall in lymphocytes either after ACTH or after doses of ACE twice as great as those effective in normal persons and in patients with infectious mononucleosis. A single individual with Addison's disease...
responded to 25 cc. of ACE by vein with a more prominent lymphocyte reduction than was observed in any of the normal subjects studied. No changes in heterophile antibody titers or titers of enteric agglutinins were noted in persons with infectious mononucleosis or normal subjects within eight hours after the administration of ACTH or aqueous ACE.

REFERENCES

CHANGES IN CIRCULATING LEUKOCYTES FOLLOWING THE ADMINISTRATION OF ADRENAL CORTEX EXTRACT (ACE) AND ADRENOCORTICOTROPIC HORMONE (ACTH) IN INFECTIOUS MONONUCLEOSIS AND CHRONIC LYMPHATIC LEUKEMIA

RICHARD H. SAUNDERS, JR. and ELIJAH ADAMS