TABLE I. Mean Percentages (± Stand. Errors) of Eosinophilic Forms in Ileo-cecal Lymph Nodes of Rats.

	Eosinophils	Eosinorrhexicytes	Eosinomeres
Controls (no pressure)	65.1 ± 4.8	.4 ± .2	34 ± 6.5
Experimentals (280 mm Hg 6 hr)	32.6 ± 3.2	15.3 ± 3.6	52.3 ± 4.4
P values*	<.01	<.01	<.02

* Probability values obtained from the distribution of Fisher's t. The 3 values are considered significant.

and lumbar lymph nodes. Examination of the colon, liver and lungs has revealed eosinophils of only the type A variety. Immature eosinophils have been seen occasionally in smears of spleen suspensions; however, these number fewer than 0.1% of the total eosinophils seen.

The question remains open as to whether an active process of eosinophilic leukocyte destruction occurs within the lymphoid organs or whether the degenerated eosinophilic forms represent bodies filtered out by the nodes. In addition, the mechanism for the breakdown of the eosinophil as seen in the rat exposed to lowered barometric pressures is still to be elucidated. Experiments are under way to determine the precise role played by the adrenal cortex in this phenomenon.

Summary. Degenerative forms of eosinophilic leukocytes are found commonly in ileocecal lymph nodes and in other lymphoid tissues of the rat. The relative numbers of these degenerative forms are increased in rats subjected to lowered barometric pressures.

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Influence of Small Doses of Heparin on Fat Tolerance Curves *in vivo* and *in vitro.** (20055)

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Hahn's(1) discovery that lipemic serum could be cleared by heparin has stimulated work on the nature of the mechanisms involved. Recently, Anderson and Fawcett(2) reported that heparin added to lipemic serum *in vitro* did not clear the serum, but when a post-heparin serum sample was mixed with the lipemic serum, clearing of the lipemic serum occurred. The material present in the post-heparin serum, was called "antichylomicronemic substance." The nature of this clearing factor has been somewhat clarified by Anfinsen and co-workers(3) who isolated a serum protein (Cohn IV-1) which in the presence of heparin and a tissue factor, resulted in the formation of the "clearing factor" (Cohn III 1, 2, 3), and they were further able to demonstrate also that once formed, the complex was activated by a protein in another serum protein fraction (Cohn III-0). Block(4) and co-workers have reported that great differences exist in the degree to which sera of different

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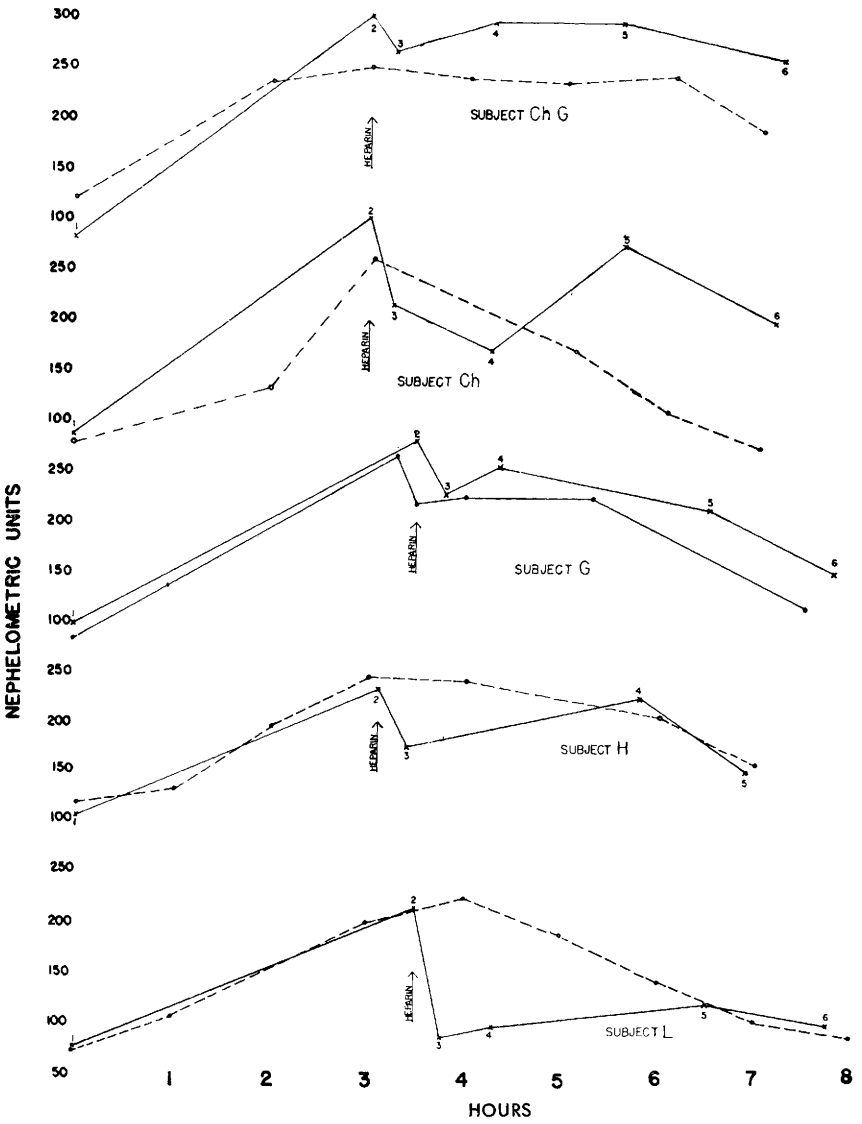

FIG. 1. The turbidity curves (×——×) of the 5 subjects who received heparin are illustrated. Except for subject G, a fat tolerance control test (○- - - -○) was available for each subject. In the instance of subject G, 2 fat tolerance tests in which heparin was used were available (×——×, ●——●) and are presented. The numerals on each curve when used in conjunction with the subjects' initials correspond to the mixture of sera in Table I. The arrows indicate the time of heparin inj. Samples No. 4 and No. 5, subject CHG, were slightly hemolyzed, causing moderate elevation of turbidimetric values.

individuals, made lipemic by a fat meal, cleared following the injection of heparin, and presented some evidence showing that in individuals suffering from arteriosclerosis, the sera were more resistant to clearing than that of normal persons.

Because of the current interest in heparin and co-factors, it was thought worthwhile to present evidence showing that the ability to develop "clearing factor" varies from person to person, and that this ability to manufacture a "potent clearing" factor is not necessarily related to the amount of heparin injected. In the course of a study of aging and arteriosclerosis, the fat tolerance test described by Moreton(5) has been used in many subjects.

The experience resulting from these studies facilitated the present one concerning the effect of heparin on lipemic sera.

Methods. Turbidity of sera was measured in a nephelometer constructed as nearly as possible like the one described by Moreton (5), using the standard of barium sulfate suspension in glycerin suggested by him. A *fat tolerance test* was carried out as follows: After an evening meal, low in fat content, the subject was asked not to smoke or eat prior to his test meal the following morning which consisted of a quantity of 20% cream calculated on a basis of surface area,† fruit juice, cereal, toast, jam and coffee. A fasting blood sample was obtained before the meal and at 3, 4, 5, 6 and sometimes seven hours afterward. Each blood sample was allowed to coagulate at room temperature, centrifuged, and the serum removed. The serum was recentrifuged, pipetted into cuvettes and the turbidity measured in the nephelometer. Peak turbidity in our patients, who were mostly over the age of 45 years, generally occurred between 3 and 4 hours after ingestion of the test meal. Total lipid content, phospholipids and total cholesterol were chemically determined on each serum sample. Since little change was noted in any of these values, except for neutral fat, the data were not included in this report.

Since it has been demonstrated that a large dose of heparin (50-100 mg) will indiscriminately clear the lipemic sera, of most individuals *in vivo,* it was decided to use a small quantity of heparin and, in an effort to achieve consistent results, relate the absolute amount given to the surface area of the subject.‡ That the size of the dose selected was good for the purpose of demonstrating individual variability in the production of clearing factor becomes evident in the results (Fig. 1).

Results. As was to be expected from previous reports, the injected heparin caused a variable degree of clearing of the serum *in vivo* in each of the 5 subjects used (Fig. 1).

It also appeared that the serum of subjects L and CH developed more potent clearing factor *in vitro,* after incubation, than did the other subjects. While in our experiments maximal clearing of serum generally occurred within a period of 45 minutes or less after the injection of heparin, one subject (CH) exhibited maximal clearing only after a 75 minute interval.

Because subject L formed such potent clearing factor *in vitro,* his post-heparin sera was mixed with that of other subjects (Table I), and it was observed that in each instance potent "clearing factor" was formed after incubation. Similarly, the post-heparin sera of subject CH proved to be quite potent when mixed with that of subject CHg and incubated (Table I). It would seem therefore that those who cleared best *in vivo* developed more potent clearing factor *in vitro,* not only as concerned their own sera, but with regard to the sera of others as well (Table I).

Since subjects L and CH cleared their sera so well *in vivo* and *in vitro* while the others did poorly, an attempt was made to correlate the clinical histories with the results. Subject H was a 32-year-old physician, mildly obese,

TABLE I. Nephelometric Units of Turbidity. The sera mixtures of the 5 subjects, L, H, G, CH and CHG are indicated with the turbidimetric values before and after incubation.

Sera mixture*	Before incubation	After incubation	
6/11/52		30 min	90 min
L1 + L2	160	160	160
L2 + L3	158	96	78
6/25/52		135 min	15 hr
L2 + L3†	132	100	77
L2 + L4	159	136	100
H2 + L4	182	155	100
H2 + L3	141	91	91
G1 + G2	245	245	245
G2 + G3	232	195	195
G2 + L3	202	136	136
		150 min	15 hr
Ch2 + CH3	265	235	250‡
Ch2 + CHG3	280	255	240
Ch4 + CHG2	255	195	172
Chg2 + Chg3	285	255	240
Chg1 + Chg4	250	—	205

* The character of the sera used for these mixtures may be found in Fig. 1.

† This mixture contained 2 vol of serum L2 to one of L3 instead of the usual equal vol mixture. Note the maximal clearing despite the disproportionate mixture.

‡ An increase in turbidity after prolonged incubation has been reported to occur occasionally.

† 162 cc 20% cream/M^2 Surface Area.

‡ Sodium Heparin U.S.P., 5 mg/M^2 Surface Area.

with a family history of vascular disease. Subject L was a paretic 50 years of age, while subjects CH and CHg were tabetics about 60 years of age. Subject G was a 57-year-old male with a history of cardiovascular disease and alcoholism. From this small group however, conclusions cannot be drawn concerning the relationship between degree of formation of clearing factor and the disease process known to be present.

Discussion. These experiments have confirmed the work of Anderson and Fawcett(2) in which they demonstrated the presence *in vitro* of an antichylomicronemic substance in post-heparin plasma samples. However, in our experiments, incubation of the sera at 37°C appeared to be necessary to initiate clearing activity *in vitro*. It is to be noted that Anderson and Fawcett used much larger doses of heparin (50 mg) than those used in our experiments, or by Block *et al.*(4).

Several problems related to the size of the single injected dose of heparin need to be answered. For example, the larger dose of heparin used by Anderson and Fawcett(2) may make incubation of sera unnecessary to initiate "clearing activity" *in vitro*. Furthermore, large doses of heparin which not only cause greater clearing of sera *in vivo*, and perhaps for a longer period of time, may also initiate greater clearing *in vitro* after incubation with the formation of more potent "clearing factor." These problems are under investigation.

Since the work of Block and his associates (4) has indicated that the *in vivo* response to a single dose of heparin may be altered in vascular disease, further studies in patients with cardiovascular disease may also demonstrate differences in the degree of formation of "clearing factor" when compared with control subjects in the same age groups.

Summary. 1. By the use of a well-standardized fat tolerance test, it has been possible to demonstrate the clearing effect of small doses of intravenously administered heparin *in vivo*. Furthermore by use of these small doses of heparin, we have demonstrated that individuals vary in their ability to clear their own sera *in vitro* after incubation. It has also been shown that an individual who clears well *in vivo* will probably clear well *in vitro*, as in the experiments described. Apparently the potent "clearing factor" formed, as for example in subject L, was very potent for the sera of other subjects who formed less "potent clearing" factor under similar conditions.

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Premature Ovulation in the Domestic Fowl following Administration of Certain Barbiturates. (20056)

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Everett(1) and Markee(2) have recently reviewed evidence indicating that neurogenic activation of the hypophysis is essential for release of ovulation-inducing gonadotrophin not only in the rabbit, which normally ovulates following copulation, but also in the rat, a "spontaneously" ovulating species. Evidence of a 24-hour rhythm in the neural mechanism was adduced in the 5-day cyclic rat when ovulation was advanced 24 hours by progesterone(3), an action of the steroid which could be blocked by atropine or dibenamine (4). This 24-hour periodicity in the neural

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