ing characteristics of human and rat serum for "physiological" quantities of cortisol and corticosterone demonstrate distinct differences in the "transcortin" systems in the blood of the two species.

Summary. Equilibrium dialysis experiments with pooled sera of rats demonstrate that the binding activity of the specific corticosteroid-binding protein ("transcortin") is higher in adrenalectomized and hypophysectomized animals than in normal controls. Rat serum at both 4°C and 37°C has a higher binding affinity for corticosterone than for cortisol, whereas in human serum at 4°C, cortisol appears to be bound more firmly than corticosterone. The "transcortin" systems of man and rat, therefore, show distinct differences.

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Chemotactic Activity and Phagocytosis of Eosinophils.* (27381)

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Neutrophils and mononuclear phagocytes have been shown to respond chemotactically to bacteria or bacterial products, but move in a random manner in the presence of injured tissue or tissue breakdown products(1). In contrast, the large accumulation of eosinophils following reexposure to antigen(2,3,4) suggests a chemotactic reaction in sensitized tissues(5,6). The present study utilizes *in vitro* preparations of inflammatory exudates to determine the factors which result in this eosinophil response.

Materials and methods. Adult C57 Bl/6 mice were immunized by 6 weekly subcutaneous injections of fluid tetanus toxoid (Lederle). Approximately 30 days after the last immunizing injection, the mice were injected intraperitoneally with 0.2 ml of 5 Lf tetanus toxoid in isotonic saline. At daily intervals, during a 10-day period, animals were killed by cervical dislocation, and autopsies performed. WBC pipets were used to withdraw from the peritoneal cavity sufficient fluid to permit total and differential cell counts, as well as preparations for living cells. The living preparations consisted of a drop of peritoneal exudate flattened between a cover glass and microscope slide and sealed with a mixture of paraffin and beeswax(7). The cells were maintained at 37°C and observed under phase microscopy for as long as 3 hours. In a few instances, the exudate was incubated for 12 or 24 hours and then examined. Zeiss lenses were used on a Unitron inverted microscope with Kohler type illumination.

Results. Observations and photographic recordings were made of more than 200

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preparations. The cells found in the peritoneal fluid of unchallenged animals consisted of mononuclear cells (96%), eosinophils (less than 3%), mast cells (1%), and very occasionally neutrophils. In the inflammatory exudate of challenged animals, the types of cells varied, depending upon length of time between injection and autopsy. Many neutrophils were observed in all preparations of exudate removed during the first 24 hours following injection, but by the second day they decreased to less than 1% of the total cell population. As the neutrophils disappeared, the proportion of eosinophils increased until by the 4th day they amounted to 30 to 50% of the cells. The eosinophils appeared to have a strong attraction to certain lymphocytes, macrophages and mesothelial cells (Fig. 1A). Commonly, 2 or more eosinophils were seen to accumulate around each cell (Fig. 1D and E). As many as 9 eosinophils have been seen to converge on a large immobile macrophage.

The eosinophils remained in contact with the mononuclear cells for periods varying from 10 minutes to an hour or more. As the eosinophils accumulated along the edges of the mononuclear cells they exhibited peculiar oscillatory or circulatory movements (Fig. 1B). Subsequent to these movements the membranes of the mononuclear cells ruptured and extrusion of cytoplasm occurred. Eosinophils penetrated into the disrupted cells and migrated back and forth through the cytoplasm (Fig. 1C), before leaving at points usually different from that of entry. During observations of several hundred of these cellular interactions, no eosinophil was seen to penetrate into the nucleus. Although in some cases the nucleus was pushed aside by the eosinophil, rupture of the nuclear membrane was never observed.

Following initial contact between eosinophils and mononuclear cells, the eosinophils would repeatedly move away, reverse their direction and return to the injured cell. In many instances their paths appeared to be circular with the eosinophils alternately pushing against and then leaving the mononuclear cells. During this process the mononuclear cells remained inactive, occasionally being pushed or dragged along by motile eosinophils. Gradual disintegration of the mononuclear cells occurred and their cytoplasm spread into the surrounding medium.

The cells which attracted eosinophils were few in number and appeared morphologically similar to those which did not. They appeared somewhat rigid, and did not show ameboid movement. Neutrophils, lymphocytes, and macrophages did not seem to be attracted to these cells although in rare instances they were associated with eosinophils within a disintegrating cell. Mononuclear cells, which had been injured by temporary pressure on the cover glass until the cell membrane ruptured, did not attract eosinophils.

By the 4th day of inflammation necrotic eosinophils were noted, and phagocytosis of eosinophils by viable macrophages was observed (Fig. 1F). Concomitantly, there was a marked drop in the proportion of eosinophils in the inflammatory exudate and a reduction of ameboid movement in the remaining eosinophils. In some preparations allowed to incubate for periods up to 24 hours, necrotic eosinophils were noted in almost every viable macrophage. As many as 10 eosinophils in various stages of disintegration were found within a single macrophage.

By the 7th day, the eosinophils had almost completely disappeared and the exudate consisted primarily of mononuclear cells with intense cytoplasmic basophilia as seen under the light microscope.

Discussion. These phase contrast preparations of living exudative cells permit observations which would be very difficult or impossible with histological or fixed smear preparations. For example, in the living preparations, eosinophils were observed to move towards certain cells and to penetrate into them. This is the first time such observations were recorded, though thousands of smears of inflammatory exudate had been previously examined. In a reexamination of the smears it was noted that eosinophils were often in close apposition to or appeared to be attached to mononuclear cells. Disintegrating macrophages were frequently surrounded by several eosinophils and, occasionally, morphologically intact eosinophils were noted within mononuclear cells. The occurrence of these

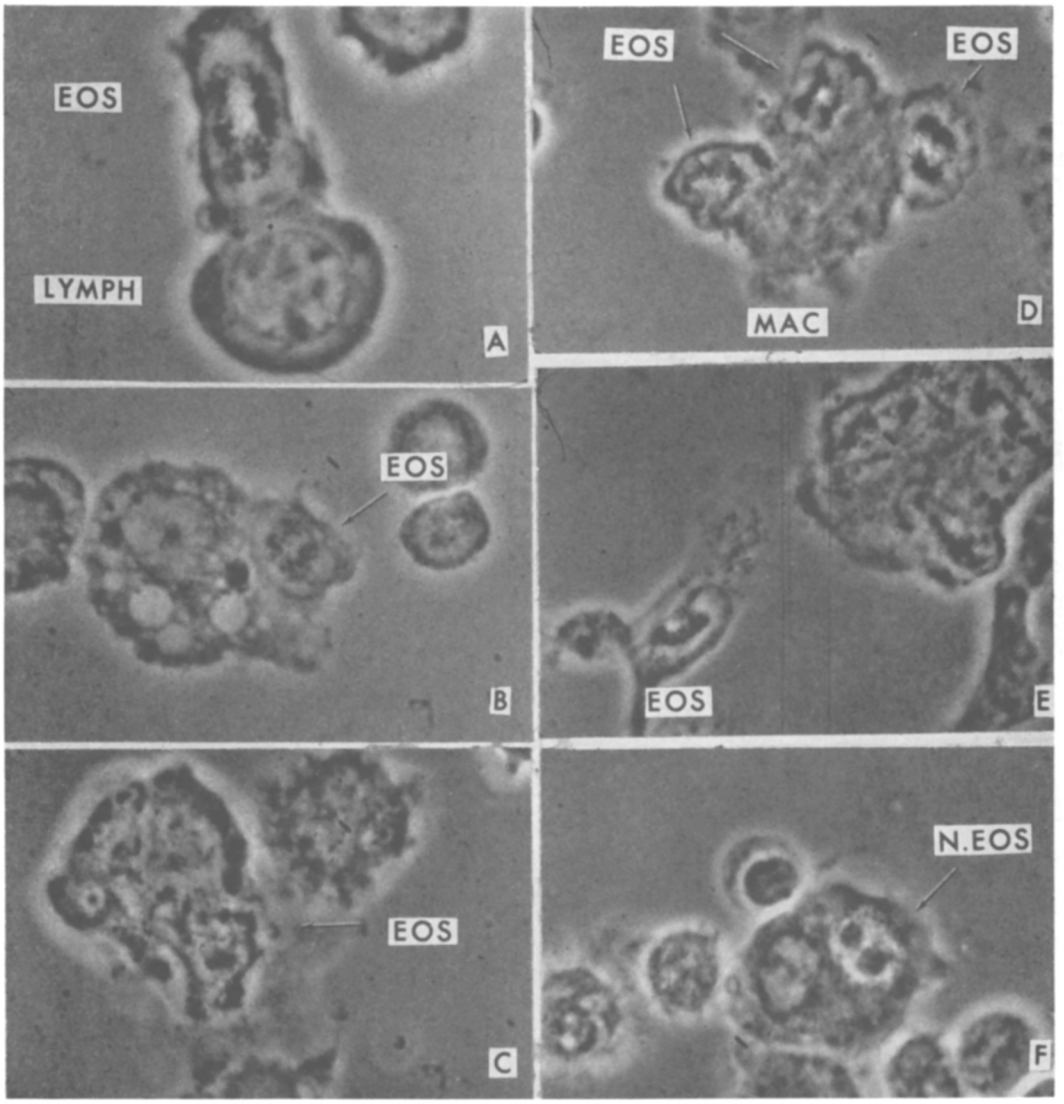

FIG. 1. Cellular interactions commonly found following a challenging inj. of tetanus toxoid. Phase contrast pictures of exudative cells. Fig. A-E during the first 3 days and F on the 6th day of inflammation. These enlargements were taken from 16 mm film strips. A) Part of a sequence showing an eosinophil moving back and forth along the edge of a medium lymphocyte. B) Eosinophil associated with a rupture in cell membrane of a macrophage which is extruding its cytoplasm. C) Eosinophil penetrating into a disintegrating macrophage. D) Three eosinophils around and within a degenerating macrophage. E) Movement of an eosinophil towards the macrophage shown in D. F) Phagocytosis of a necrotic eosinophil.

cell groups in smear preparations indicates that the attraction of eosinophils to certain cells must occur *in vivo* as well as *in vitro*.

The ameboid movement of eosinophils towards certain cells or cell masses (Fig. 1E), and the circular motion made by the eosinophil as it repeatedly pulls away and returns to the same mononuclear cell indicates that an attraction exists between the 2 cells. Since the eosinophils appear not to penetrate into the cell nucleus, it is presumed that the eosinophilotactic material must reside primarily in the cytoplasm. Mononuclear cells disrupted *in vitro* by mechanical means failed to attract nearby eosinophils. In other experiments(10), exudative cells killed by freezing

and thawing failed to produce a local eosinophilia or otherwise to attract eosinophils *in vivo*. Thus, cell injury or disintegration does not appear to be the factor attracting eosinophils. In experiments in which radioactive tetanus toxoid was injected into immunized animals, examination of the autoradiograms of the exudate indicated that the eosinophils were associated primarily with mononuclear cells which contained radioactive material rather than with free antigen or antigen-antibody complex(10).

These observations indicate that reinjection of an antigenic material affects specific mononuclear cells causing them to attract eosinophils. The antigen presumably combines with some material in the cell. It is not known whether this material is antibody or some other cellular component. Since the eosinophilia does not occur when antigen is injected into passively immunized mice, factors other than union of antigen and antibody must be involved(3,12).

The attraction of eosinophils to mononuclear cells occurs between the 1st and 3rd day after the challenging injection. Beginning on the 4th day of inflammation, eosinophilotactic activity diminished, and phagocytosis of eosinophils by viable macrophages is observed, as previously reported(3,9,10,12).

These data and those of similar experiments suggest that the role of the eosinophil may be to transfer material from cells injured by the antigen to viable cells capable of taking part in the immune response. They are consistent with the theory that eosinophils play a role in initiation of antibody production during the secondary response(3,12). These observations clearly indicate that the cellular responses during an inflammation vary not only in number and types of cells reacting, but also in the physiological attraction and reaction of cells to each other. These cellular interactions may be of fundamental importance in sensitization and immunity.

Summary. Eosinophils were demonstrated to be chemotactically attracted to certain mononuclear cells in an inflammatory exudate produced by a challenging injection of tetanus toxoid. The accumulation of eosinophils around specific lymphocytes, macrophages and mesothelial cells was followed by a rupture of the cell membrane and penetration of the eosinophils into the mononuclear cell. The eosinophils eventually move away from the disintegrating cells and, beginning on or about the 4th day of inflammation, are rapidly engulfed by viable macrophages.

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