since they occur mainly in different fractions of the cell.

That steroid hormones may affect enzymes is well established, and the variety of quantitative effects includes both in vivo and in vitro activation and inhibition (*7, 8*), as well as in vivo increase in production (*9*) of enzymes. However, qualitative enzyme differences between the sexes, other than those occurring in the sex organs, have not been described. Total esterase activity of mouse kidney has been reported to be no different in males and females (*7*). The mechanism of the action of testosterone in our results cannot be determined without further data. The time course and the absence of in vitro effect imply an indirect route, perhaps through stimulation of the production of enzyme. In this connection, Zalokar (*10*) has suggested that steroid hormones may function as gene activators to induce synthesis of enzyme; more recently, it has been shown (*11*) that testosterone accelerates the rate of incorporation of valine from soluble RNA into ribosomal protein.

Whether the male kidney esterase has any physiological activity is not known. It could represent simply a metabolic side effect, but this seems unlikely in view of its strong activity in the test system. A possible role is that it is an adaptive enzyme that functions in the excretion of male steroid products (*12*).

CHARLES R. SHAW
ANN L. KOEN
Hawthorn Center, Northville, Michigan

References and Notes

1. R. L. Hunter and C. L. Markert, *Science* **125**, 1294 (1957); C. L. Markert and F. Moller, *Proc. Natl. Acad. Sci. U.S.* **45**, 753 (1959).
2. M. L. Bach, E. R. Signer, C. Levinthal, I. W. Sizer, *Federation Proc.* **20**, 255 (1961).
3. S. L. Allen, *Ann. N.Y. Acad. Sci.* **94**, 753 (1961).
4. D. Schwartz, *Proc. Natl. Acad. Sci. U.S.* **46**, 1210 (1960).
5. R. E. Tashian and M. W. Shaw, *Am. J. Human Genet.* **14**, 295 (1962); C. R. Shaw, F. N. Syner, R. E. Tashian, *Science* **138**, 31 (1962).
6. A. G. E. Pearse, *Histochemistry* (Little, Brown, Boston, 1961).
7. W. E. Knox, V. H. Auerbach, E. C. C. Lin, *Physiol Rev.* **36**, 164 (1956).
8. K. L. Yielding and G. M. Tompkins, *Proc. Natl. Acad. Sci. U.S.* **46**, 1483 (1960).
9. H. L. Segal, D. S. Beattie, S. Hopper, *J. Biol. Chem.* **237**, 1914 (1962).
10. M. Zalokar, in *Control Mechanisms in Cellular Processes*, D. Bonner, Ed. (Ronald, New York, 1961).
11. S. Liao and H. G. Williams-Ashman, *Proc. Natl. Acad. Sci. U.S.* **48**, 1956 (1962).
12. Supported in part by a grant from the Scottish Rite Foundation for Research in Schizophrenia. We thank Dr. Morris Foster, Laboratory of Vertebrate Zoology, University of Michigan, for certain mouse strains, and Lucile Setter and Mary Conley for technical assistance.

10 January 1963

Antigenic Material: Persistence in Hypersensitive Cells

Abstract. *Radioactivity derived from injections of tritiated tetanus toxin was observed in lymphocytes and macrophages for as long as 9 months after injection into immunized mice. These labeled cells became highly vacuolated and attracted eosinophils and lymphocytes when the animals were reinjected with specific antigen.*

Purified tetanus toxin was tritiated by the Wilzbach procedure and injected intraperitoneally into mice which had been previously immunized with tetanus toxoid. Autoradiograms were made of the inflammatory exudate to determine the types of cells which contained radioactive material. Experiments reported earlier indicated that the type of labeled cells varied depending upon the length of the interval after injection (*1–3*). In experiments reported at this time, attempts were made to determine whether antigenic material persists in specific cells after the acute stages of inflammation have subsided, and if so, whether these cells were capable of responding to a reinjection of antigen.

The cytoplasm of cells in the peritoneal cavity, spleen, and bone marrow of animals autopsied 10, 30, 60, and 270 days after injection of the tritiated toxin showed radioactivity. The types of labeled cells varied.

In the animals autopsied 10 days after injection of tritiated toxin, lymphocytes (Fig. 1) and macrophages (Figs. 2 and 3) showed radioactivity. In animals autopsied at 30, 60, and 270 days, the radioactive material was present primarily in large macrophages and multinucleated cells similar to the cell shown in Fig. 4. In most of the animals the radioactivity was present in the form of clumps associated with basophilic material (arrows on Figs. 2 and 3).

Other animals were reinjected intraperitoneally with either nontritiated tetanus toxoid or diphtheria toxoid and autopsied 1, 3, 5, 7, and 10 days later. A summary of the number of labeled cells and grain counts is shown in Table 1. Only slight differences occurred between the two groups of animals in the number of labeled cells and the average number of grains. However, marked differences did occur in the morphological appearance of the labeled cells. In the animals rechallenged with tetanus toxoid, approximately 50 percent of the labeled cells appeared to be swollen, to be highly vacuolated, and to have indistinct cell membranes (Figs. 5–7). Eosinophils and lymphocytes were commonly attached to these necrotic cells so that rosettes were formed (Figs. 6 and 7).

In the animals injected with diphtheria toxoid, the labeled cells were seldom vacuolated or swollen, and eosinophil rosettes were rarely observed. Lymphocytes, however, were frequently observed around both labeled and unlabeled macrophages.

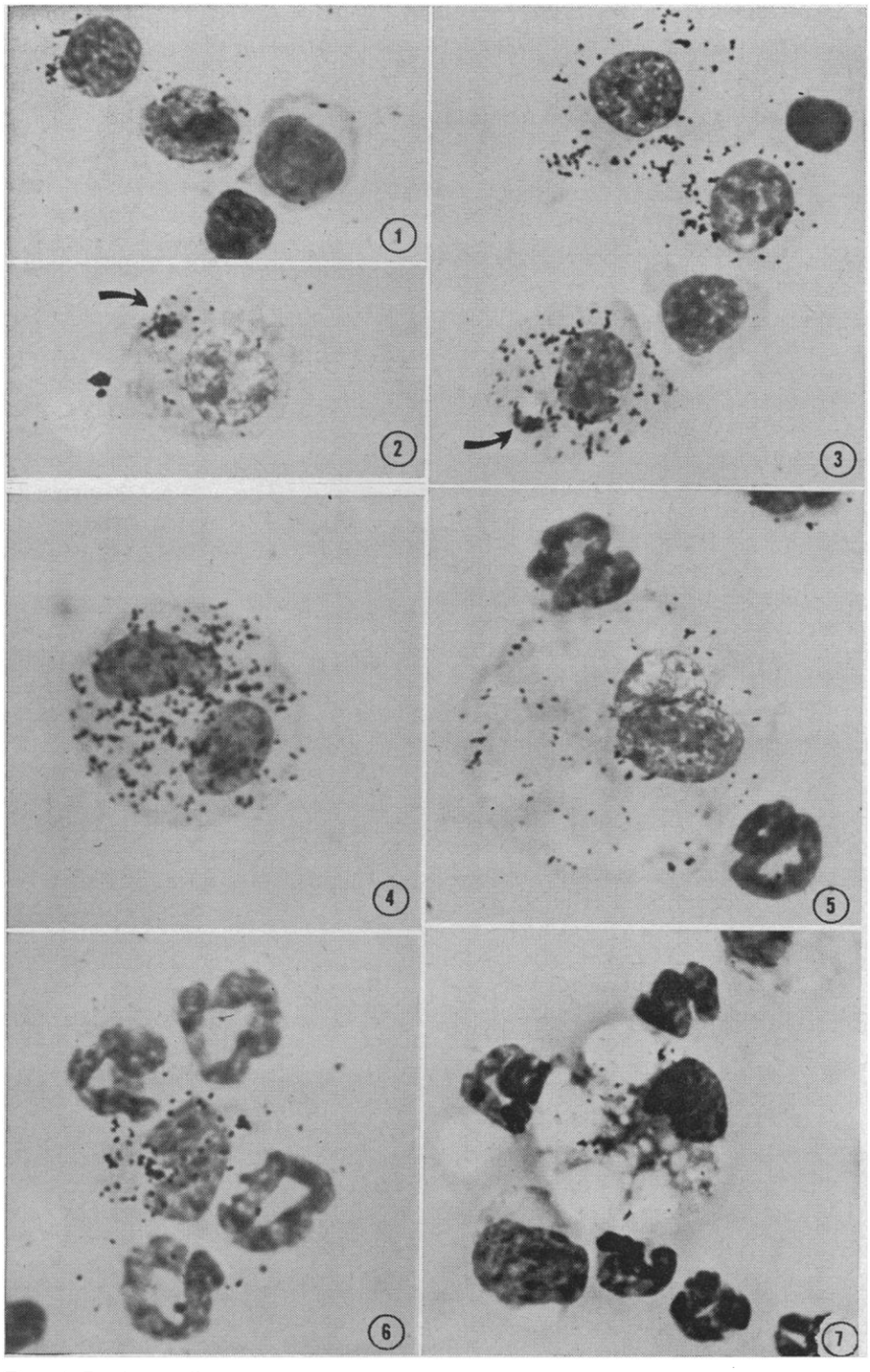
The data suggest that antigenic components associated with cytoplasmic basophilia persist in macrophages for prolonged periods after injection. The presence of this antigenic material makes these cells hypersensitive and they become highly vacuolated when reexposed to specific antigen.

Earlier experiments with radioactive antigen demonstrated that after the inflammation had subsided, there was a marked decrease in the number of labeled cells in the inflammatory area, and an increase in labeled cells in the spleen, lymph nodes, and bone marrow (*3*). Reinjection of a nonradioactive antigen resulted in an increase in labeled mononuclear cells and a reappearance

Table 1. Grain counts over labeled inflammatory cells at various periods after injection of radioactive antigen. The animals were sensitized with tetanus toxoid (nine injections), and tritiated tetanus toxin (three injections), and challenged with tetanus toxoid and diphtheria toxoid.

Days between sensitization and autopsy	No. of mice	Cells scored	Labeled cells (%)	Average grain count above background*
Challenge: tetanus toxoid (2 Lf per 0.2 ml)				
10 to 20	12	23,500	9.0	33.2
30 to 40	12	26,171	6.3	38.0
60 to 70	13	33,500	2.3	18.3
Challenge: diphtheria toxoid (2 Lf per 0.2 ml)				
10 to 20	6	11,600	8.1	39.8
30 to 40	6	10,000	7.0	32.3
60 to 70	9	15,500	2.5	15.8

* Average background grain was 3 per 100 μ^2. A few labeled cells were also observed 270 days after sensitization, but the number was too low to provide a meaningful percentage.



Figs. 1–7. Autoradiograms of inflammatory cells taken from the peritoneal fluid of mice at various times after injection of tritiated tetanus toxin. Fig. 1. Labeled lymphocytes 10 days after injection. Figs. 2 and 3. Labeled macrophages 10 days after injection. The radioactive material is often associated with basophilic material in the cytoplasm (see arrows). Fig. 4. Highly labeled binucleated cell from the inflammatory exudate produced by injection of diphtheria toxoid 30 days after injection of radioactive tetanus toxin. Fig. 5. Highly labeled binucleated cell from an animal which had been rechallenged with tetanus toxoid 30 days after injection of radioactive tetanus toxin. This cell is highly vacuolated and two eosinophils appear to be attached to it. Fig. 6. Labeled disrupted macrophage and four attached eosinophils. This rosette is from a mouse rechallenged with tetanus toxoid 10 days after injection of radioactive tetanus toxin. Fig. 7. Labeled cell from an animal which had been rechallenged 270 days after injection of radioactive tetanus toxin. This highly vacuolated cell had three eosinophils attached.

of radioactivity in the inflammatory area (*4*). It would appear that the migration of these labeled cells into the inflammatory area initiates a series of reactions when the cells come in contact with specific antigen. The cells become swollen and release biologically active substances which produce a chemotactic response from the eosinophils (*2*, *5*). The accumulation of vacuolated cells with associated eosinophils occurs before the formation of measurable serum antibody, and the time of antibody production corresponds to the time the eosinophils and the fragments of the hypersensitive cells are engulfed by macrophages (*6*). This suggests that these cellular reactions are part of the processes causing delayed hypersensitivity and the formation of antibody (*7*).

ROBERT S. SPEIRS
Department of Anatomy, Downstate Medical Center, State University of New York, Brooklyn 3

References and Notes

1. R. S. Speirs, in *Tritium in the Physical and Biological Sciences*, International Atomic Energy Agency (Paul Gerin, Vienna, 1962), vol. 2, p. 419.
2. ———, symposium on *Conceptual Advances in Immunology and Oncology* (Univ. of Texas Press, Austin, in press).
3. R. S. Speirs, and E. E. Speirs, *J. Immunol.*, in press.
4. ———, in preparation.
5. R. S. Speirs, and Y. Osada, *Proc. Soc. Exptl. Biol. Med.* **109**, 929 (1962).
6. R. S. Speirs, *Nature* **181**, 681 (1958).
7. Supported by a grant from the Atomic Energy Commission (30-1)-2414, and contract I-95 with the Health Research Council of the City of New York.

14 January 1963

Crystal Structures of Titanium, Zirconium, and Hafnium at High Pressures

Abstract. *At high pressures, as determined by x-ray analysis, titanium and zirconium metal have a distorted, body-centered-cubic structure. This phase persists on pressure release. The normal hexagonal close-packed structures are recovered when the metals are heated. An electronic shift must occur in the transition. Hafnium metal showed no such transition.*

The isoelectronic transition elements [$nd^2(n+1)s^2$] titanium, zirconium, and hafnium are all hexagonal closed-packed (h.c.p.) metals at room temperature and pressure, while at high temperatures they adopt a body-centered-cubic (b.c.c.)