Summary. When increasing amounts of thyroxine are administered to rats otherwise given an invariable dose of TRF, a dose of thyroxine is found to completely inhibit the TSH-releasing activity of that dose of TRF. Conversely, when increasing amounts of TRF are administered to animals pretreated with an invariable quantity of thyroxine known to inhibit the TSH releasing activity of several doses of TRF, a dose of TRF is eventually reached that will overcome the inhibitory effect of that amount of thyroxine. Neutron activation of a highly purified preparation of hypothalamic TRF failed to reveal presence of iodine in the TRF molecule.

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Myocardial Lesions: Spontaneous Development in Captive Ground Squirrels.* (32052)

JEROME P. SCHMIDT AND JACK A. REHKEMPER†
(Introduced by J. D. Fulton)
USAF School of Aerospace Medicine, Brooks Air Force Base, Texas

This investigation stems from the earlier incidental discovery of myocardial lesions in untreated, apparently healthy captive ground squirrels(1,2) and monkeys(3). It was suggested that the lesions observed may have resulted from naturally acquired microbial infections or from the stress of captivity. The duration of the captive period was not reported but is presumed to have been several months. This is the first report of studies in which tissues from noncaptive animals were available for comparison with those from animals held captive for definitive periods.

Methods. Hearts of 29 arctic ground squirrels *(Citellus undulatus)* shot or captured in Central Alaska were examined. Captured animals were individually caged and isolated from other animals and from known sources of infection. They were provided with water and a nutritionally adequate diet which included fresh, leafy vegetables and apples daily. The caged animals were sacrificed with pentobarbital sodium solution after 2, 3 or 4 weeks in captivity. Hearts of all animals were fixed in neutral 10% formalin. Representative portions of cardiac muscle were imbedded in paraffin, sectioned at 6 μ and stained with hematoxylin and eosin, periodic acid-Schiff, Brown-Brenn, or Masson's trichrome. These preparations were then studied by light microscopy.

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† Present address: 2794 USAF Dispensary, Kelly Air Force Base, Texas.

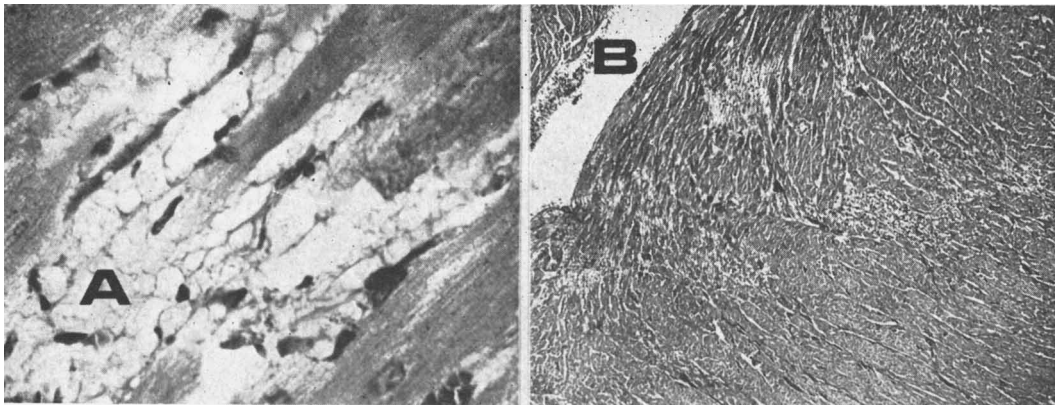

FIG. 1. Myocardium of ground squirrel. Hematoxylin and eosin. A. Early noninflammatory degeneration. Note adjacent viable muscle fibers. 400×. B. Several areas of focal degeneration. 34×.

Results. Grossly, there were no detectable lesions in the fixed tissues. Microscopic examination revealed no definitive myocardial lesions in any of the specimens from animals shot in the field. However, 5 of 7 hearts from animals held captive for 2 weeks exhibited changes varying from tiny focal areas of muscle necrosis to larger areas showing a paucity of muscle fibers and shrunken hyperchromatic nuclei (Fig 1).

Of 7 hearts from squirrels caged for 3 weeks, 3 had necrotic lesions similar to but less extensive and fewer in number than those observed in hearts of animals held for 2 weeks. Each of the 7 hearts from animals held captive for 4 weeks had a few foci of degenerate muscle fibers. Some areas of scarring and a moderate degree of mineralization were also observed.

Discussion. Myodegenerations apparently occur in animals spontaneously and under a number of experimental conditions(4). Our histologic findings reveal that spontaneous myocardial lesions may develop in ground squirrels held in captivity. While no definitive necrotic lesions were observed in any of the hearts from animals destroyed in the field, they were found in hearts of captive animals. Heart tissues from animals caged for 2 weeks exhibited the most evident destructive necrotic lesions. Lesions decreased in number and size in the groups held captive 3 and 4 weeks. We observed no inclusion bodies or other evidence of virus infection but no isolation attempts were made. The possibility of an activated latent virus infection cannot be excluded.

The onset of formation of these spontaneous lesions has not been determined. Our data indicate that definitive lesions may be found following 2 weeks of captivity and suggest that repair of these degenerative changes occurs in the succeeding periods. This finding implies that active lesions may not be expected in animals maintained in captivity for extended periods. However, it is possible that animals, once reconciled to captivity, may be subjected to a new "stress" in the course of their selection and preparation for a specific experimental procedure. This factor could account for the lesions observed by others in animals presumed to have been held for longer periods.

The findings reported here are of significance to investigators using captured animals since animals thus affected are not considered to be normal. It is unlikely that ground squirrels are unique in their response to captivity.

Summary. Microscopic examination revealed that hearts of arctic ground squirrels killed in the field (noncaptive) were free of myocardial lesions but numerous areas of focal necrosis were found in the hearts of animals held captive for 2, 3, or 4 weeks. The etiology of these degenerative changes may be associated with the stress of captivity, but remains to be determined.

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Passive Cutaneous Anaphylaxis Induced in Mice with Rabbit 5S Antibody Fragments.* (32053)

FRANCIS B. CASEY, JR.† AND SEI TOKUDA†
(Introduced by L. C. McLaren)
Department of Medical Microbiology, College of Medicine, University of Vermont, Burlington

When γG-immunoglobulins (7S) molecules are treated with cysteine activated papain or with pepsin some of their original serological activities are retained.

Cysteine-activated papain splits rabbit and human γG-immunoglobulin molecules into 3 fragments which together account for 90% or more of the original molecule(1). Two of these fragments are almost identical and are called Fab fragments(2). Each Fab fragment has a sedimentation constant of 3.5S, contains a single antibody-combining site and behaves as a "univalent" antibody. With regard to specificity and combining power, the antibody-combining sites of the Fab fragments appear to be the same as those of the original intact molecule, indicating that the antibody-combining sites are not appreciably altered by the enzyme(3,4). The third component is called the Fc fragment. This portion does not have any antibody-combining sites.

Pepsin reduces γG-immunoglobulins to about 2/3 of their original size(1,4). The pepsin-digested material has a sedimentation constant of 5S and has two antibody-combining sites per molecule. The 5S antibody fragments are believed to consist of 2 Fab fragments joined together by a single disulfide bond. The portion destroyed by enzymatic digestion corresponds to the Fc fragment. The 5S fragments derived from pepsin digestion behave like bivalent antibodies capable of exhibiting agglutinating or precipitating activities.

Fragments lacking the Fc fragment, whether obtained by either papain(5) or pepsin(6) treatment, do not sensitize guinea pig skin for the passive cutaneous anaphylaxis (PCA) reaction. Although aggregated γ-globulin as well as aggregated Fc fragments cause cutaneous reactions in guinea pigs and fix guinea pig complement, aggregated Fab fragments do not(7). Brambell *et al*(8) demonstrated that while the Fc fragments cross the placental barrier, the Fab fragments do not. It is therefore believed that the Fc fragment contains the site(s) responsible for: a) skin attachment in the cutaneous reaction; b) complement fixation and c) placental transmission of antibody(1).

It is generally accepted that the antibodies responsible for systemic anaphylaxis must "fix" to host tissues at certain critical sites in order to trigger the toxic reaction(9). However, Kind and Goodman(10) reported that pepsin-digested rabbit γ-globulin fractions (presumably containing the bivalent, 5S antibody fragments, which lack the sites responsible for tissue fixation) sensitize the mouse for passive systemic anaphylaxis.

The purpose of our investigation was an attempt to resolve the apparent discrepancy between the inability of 5S antibody fragments to elicit PCA in the guinea pig and

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† Present address: Dept. of Microbiology, School of Med., Univ. of New Mexico, Albuquerque.