diet. 4. This latter finding, in conjunction with the results of urinary analysis of ionic constituents, indicated that the increased excretion of organic acid is not a consequence of an altered metabolism that decreases body fat deposition but is a renal acid-base mechanism for neutralization of increased urinary calcium, an increase resulting from the enhancement of gastrointestinal absorption of calcium by incompletely digested carbohydrates.

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Leucinamidase Activity Associated with Influenza-Parasite Complexes in Pigs.* (26412)

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Activity of amino acid amidases is increased in homogenates prepared from bovine kidney monolayered tissue cultures when infected with the virus of infectious bovine rhinotracheitis virus. Leucinamidase was the most active of the 5 enzymes studied(1). Amide-N^{15} from either leucinamide or glutamine was incorporated into cellular nucleic acid in both normal and virus infected cells but activity was greater in the infected cells (2). Studies have been extended to evaluation of serum leucinamidase activity in pigs. The enzymic activity in serum of pigs which were infected with swine influenza virus, *Ascaris suum,* and lungworms (*Metastrongylus pudendotectus* and *M. apri*) which harbor influenza virus is herein reported.

Materials and methods. One week-old pathogen-free, colostrum-deprived pigs housed in modified Horsfall-Bauer isolation units as previously described were used as the experimental host(3). Eight littermate pigs were utilized in pairs for each of 4 experimental conditions. These were: 1) Uninoculated controls; 2) Inoculated with Shope's strain 15 of swine influenza virus; 3) Fed infective eggs of *Ascaris suum;* and 4) Fed infective larvae of swine lungworms originating from influenza-infected pigs. Fifteen days after the lungworm larvae were given to the pigs *Ascaris* eggs were fed to provoke the masked influenza virus(4). Methods used for preparation and inoculation of virus and parasites(5,6,7) and for evaluation of

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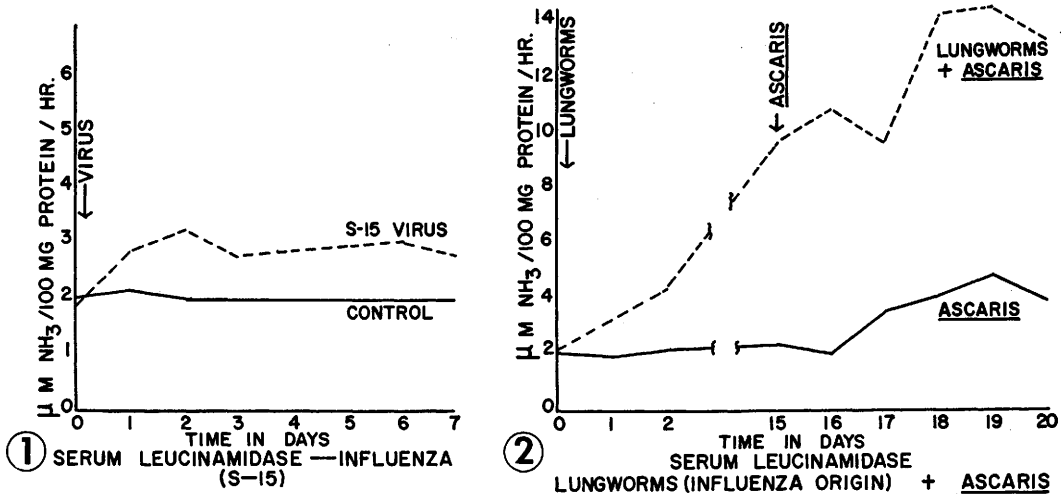

FIG. 1. Leucinamidase activity in serum of normal pigs and pigs infected with swine influenza (S-15) virus.

FIG. 2. Leucinamidase activity in serum of pigs infected with *Ascaris suum* larvae and pigs infected primarily with lungworm larvae of influenza origin and secondarily with *A. suum* larvae.

leucinamidase(8) have been described. Serum was obtained from blood drawn from the anterior vena cava.

Results. Leucinamidase activity remained relatively constant in the uninfected control pigs at approximately 2 μ M of NH_3/hour/100 mg serum protein (Fig. 1). Activity was slightly increased in the influenza infected pigs with values near 3 μ M. Body temperatures remained normal in the uninoculated pigs but were slightly increased in virus infected pigs. At necropsy 7 days after infection, lungs of control pigs were normal whereas the lungs from influenza virus infected pigs showed typical pneumonic areas.

The pigs which received embryonated *Ascaris* eggs on the 15th day showed amidase activity similar to the uninoculated controls up until 2 days after feeding *Ascaris* eggs (Fig. 2). A noticeable rise in activity began at the time larval migration was at its height. Temperatures remained normal. Lungs at necropsy showed only hemorrhages typical of *Ascaris* larval migration.

Pigs inoculated with lungworms of influenza origin showed the greatest amount of serum leucinamidase activity(Fig. 2). Typical swine influenza lesions and lungworms were present in the lungs at necropsy. Swine influenza virus was isolated from the lungs by egg inoculation and specifically identified by serum neutralization test. Increased leucinamidase activity in these pigs appears to be accumulative from the concerted effect of an established lungworm infection, migrating *Ascaris* larvae, and an active swine influenza virus infection.

Discussion. Increased leucinamidase activity in swine appears to be associated with cellular damage whether caused by virus or parasites. Other observations in this laboratory (unpublished) show little or no association of increased activity to febrile response. Virus pneumonia of pigs (VPP), which is a chronic respiratory disease, induces no temperature reaction yet elicits increased leucinamidase activity. Contrarily, swine edema disease which causes severe endothelial and myocardial damage, induces temperatures 107°-108°F but leucinamidase activity increases rapidly to 6-8 μM before temperature increases are evident. Hog cholera follows a similar pattern.

An association of leucinamidase to host resistance is implied in these experiments. Lungworms of influenza origin are incapable of producing influenza in pigs without some external stimulus. Migrating *Ascaris suum* larvae with associated increased leucinamidase activity served as a provoking stimulus

to free lungworm-bound influenza virus. Aqueous extracts of *Ascaris* also stimulate increased leucinamidase to provoke the virus (5). This eliminates tissue damage by migrating larvae as necessary to provoke influenza infection.

No tests have been made on serums from pigs infected experimentally with pathogenic bacteria or leptospira. These obviously must be made.

Enzyme activity is a sensitive health indicator. In our laboratory, 2-3-day-old pigs, infected with *Ascaris* larvae, show a rise followed by a partial decline in leucinamidase activity when the larvae pass through the intestine, when they pass through the liver (most marked), and again when they pass through the lungs. Established base lines of serum enzyme activity might be useful indicators of health in other species. Amino acid amidase activity might indicate latent infections or parasitism which would eliminate potential hosts from experiments that would be voided by a complex etiology.

Summary. A moderate increase in serum leucinamidase activity occurred in colostrum-deprived pathogen-free pigs experimentally infected with swine influenza virus. Activity also increased during migration of *Ascaris suum* larvae through the liver and again when the larvae reached the lungs. Greatest leucinamidase activity was stimulated when influenza virus was provoked from its latent lungworm state by migrating *A. suum* larvae.

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Serum and Urine 17-Hydroxycorticosteroid Levels Following Small Oral Doses of Synthetic Analogs of Hydrocortisone.* (26413)

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Earlier reports from these laboratories enumerated changes in body fluid 17-hydroxycorticosteroid (17-OHCS) levels following oral administration of synthetic analogs of hydrocortisone(1,2). Parotid fluid steroid findings have reliably reflected serum and urine results. However, the *magnitude* of steroid response differed with each drug, even though all doses were 50 mg. The present study was designed to investigate this variability in steroid response by employing smaller doses of hydrocortisone analogs.

Materials and methods. Volunteer subjects were 281 young adult male airmen, all judged physically qualified for military service by recent medical examination. Diet, environmental exposure, times of arising and retiring, and other conditions were virtually identical in all instances. Each day, 16 subjects were randomly divided into experimental and control groups, and these groups further divided into 4-hour and 7-hour groups. At 0800, blood was collected by venipuncture and the serum retained. A force-voided urine specimen was discarded. A second blood sample was collected at 1200 or 1500 hours, *i.e.*, either 4 or 7 hours after the initial sampling. Urine was collected over the respective 4 or 7-hour periods. The drugs

* The opinions expressed herein are those of the authors and are not to be construed as representing USAF policy.