

BODY OF REPORT

SEATO Medic Study No. 84 A Study of the Ecology of the Gibbon and
Tree Shrew in the Field and Laboratory

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Division of Medical Research Laboratories

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Principal Investigator: Lt Colonel John H. Morris, VC

Associate Investigators: Captain Sylvanus W. Nye, USAF, MC
Captain Richard O. Spertzel, VC
Norman C. Negus, Ph.D.
Dhira Sonakul Comer, MD

Assistant Investigators: Captain Philip Z. Sobocinski, MSC
Major Dale E. Wykoff, MSC
SSG Eugene F. Clary, (E-6)
SSG Cecil Pugh, Jr., (E-6)
Prayot Tanticharoenyos, DVM

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Objective: To evaluate the potential of the gibbon and tree shrew for use as laboratory primates in the study of infectious diseases, drug toxicity, nutrition, physiology and biological behavior. To evaluate and seek solution to any difficulties these animals may present as experimental animals. To define norms of anatomy, health and nutrition under both field and laboratory conditions. To ascertain the natural diseases, ecto and endoparasites of these animals. To

determine the ability of these animals to be colonized in Thailand and other parts of the world.

Description: The tree shrew (*Tupaia glis*) a small hardy primate found in abundance in Thailand is receiving increasing attention in the United States as a potential research animal. This animal, one of the most primitive primates in the evolutionary scale, would be a desirable research animal if it could be adapted to laboratory conditions. The tree shrew would hopefully have most of the biological advantages of the primate and because of its size and hardiness, the housing and care advantages of a smaller animal. Attempts at breeding these animals in captivity have met with very limited success.

The white handed gibbon (*Hylobates lar lar*) is widely distributed in Thailand. These apes are high on the primate tree and as such are excellent subjects for behavioral studies. Published reports by Carpenter (1940) and Kawamura (1958) illustrate the life cycle, individual behavior, social patterns, the communication and other aspects of the natural life of this animal. Attempts at breeding in captivity have met with very limited or no success. The approach to this project must depend on our understanding of fundamental inter-relationships of these animals and their interactions with their environment. Our understanding of the interactions of these animals in field situations may determine the survival of the animals in conditions suitable for research purposes. The project will be divided into two stages: pilot study and detailed study.

1. Gibbon (*Hylobates lar lar*): The pilot study will begin in field areas where the gibbons can be obtained. Thirty-five (35) gibbons will be obtained and studied under caged conditions. A physical examination will be given at time of receipt. Stool samples will be acquired for bacteriological, virological, and parasitological examination upon receipt and on days 2 and 3. Blood will be drawn at time of receipt for hematological and parasitological examination. Serum will be obtained and frozen for future reference. Cultures for respiratory flora (virological and bacteriological) will be obtained at time of receipt.

Animals will be caged individually for thirty (30) days for conditioning. Once animals have been found free of *Shigella sp.* and internal parasites, have adapted to the diet, and have been found to be otherwise normal, they will be caged under various cage conditions. Psychological characteristics of the animals maintained under these conditions will be observed and recorded daily. Stools, rectal swabs, and blood will be collected from the animals monthly for laboratory examination. Blood pressure, pulse, respiration and weight will be recorded at this time. Animals will be tuberculin tested every three months.

Sick animals will be removed from cages and isolated from the colony. Necessary laboratory examinations will be performed as indicated. All dying animals will be necropsied, and subjected to a complete histopathological work up. Swabs of liver, lung, spleen and intestine will be sent for micro-biological study. Serial sacrifice of two (2) animals from one cage of four, two (2) animals caged

together and one (1) individually caged will be accomplished on Day 30, Day 240 and Day 360.

2. Tree Shrew (Tupaia glis): Young tree shrews will be obtained. Some will be used to study ideal cage size, type and number of animals per cage. Some will be used for biochemical, histopathological, microbiological and parasitological studies. Caged animals will be observed daily. Various diets will be fed individual groups. Sick animals will be removed and isolated from the colony. Treatment will be administered as symptoms dictate. Dead animals will be necropsied. Sacrifice of newly purchased and conditioned animals will be made for biological studies. Necessary fecal, blood, serum and tissue samples will be collected from sick, dead or sacrificed animals. Other studies will be performed as necessity dictates or opportunities allow.

Progress:

1. Gibbon - Plans are being made, cages and equipment are being acquired.

2. Tree Shrews - Two shrews (one male and one female) per cage were each given (GBL Baking Co.) rat and mouse diet, simian diet and dried dog meal (various companies) to see if shrews could subsist on any of these diets. The former two (2) pairs of shrews survived 4, 13; 6, 12; respectively. One male and one female tree shrew were maintained on dog food for 30 days before being killed for laboratory studies (designated TS 1 and TS 2). One male shrew maintained on fruit fortified with vitamins for 20 days, was gradually losing weight and was changed to dog food and has regained weight and remained in apparent good health for the last 66 days. One male and one female have been maintained on dog food for 85 days and have remained in excellent physical condition. Based on these unexpected, remarkable results with dog food, this diet has been used for the remainder of the studies. Twenty seven (27) females and twelve (12) males have been maintained on this diet for 5 months.

Five cage designs are being investigated initially. All compare favorably in regard to health and well being of the animals. Nesting boxes or solid partitioning offering the shrews an opportunity to isolate themselves in a darkened area appear to be essential. The cages presently in use are as follows:

a. Aluminum construction with galvanized wire sides top and bottom. Dimensions 48" x 24" x 24" with nest boxes 7" x 15" x 8" and removable wire partition.

b. Galvanized wire construction dimensions 24" x 29" x 16" with nest boxes 7" x 15" x 8".

c. Aluminum construction, solid back, sides and top galvanized wire front and bottom dimensions 24" x 29" x 16" with nest boxes 7" x 15" x 8".

d. Galvanized metal, wire and wood construction double compartment. Dimensions 48" x 24" x 18". Built in nest box constructed of wood.

e. Stainless steel 24" x 24" x 8" with stainless steel wire top.

Limited data has been obtained which indicates that all of the cages are satisfactory for housing these animals. Cage C appears to be quite suitable for housing one pregnant female prior to parturition or one male and two female non-pregnant shrews. Mating and conception has occurred in all designs except cage E. Water is supplied via water bottles and drinking tubes similar to those used for other laboratory animals. Water consumption is not impaired and this seems to be a very efficient method of supplying it. About 5% mortality occurs within the first 30 days of captivity. After a 30 days stabilization period no deaths occur.

Five tree shrews have become pregnant in captivity and five pregnant shrews were purchased. One of the former group and three (3) of the latter group have given birth to 1, 2, 2, and 3 babies respectively. Two of these litters are doing fine after 5 days. Of the other two one failed to nurse the young the other ate her babies.

Fifteen (15) male and thirteen (13) female tree shrews have been sacrificed for laboratory studies. Four shrews died and tissues were submitted for pathology only. The hematology, biochemistry results are summarized in Tables, I, II and III.

Coliform, Proteus sp, paracolon sp, Pseudomonas sp, and Strep fecalis were found 100%, 90%, 48%, 62% and 71% of intestinal specimens respectively. In addition, aeroboeter sp, klebsiella sp, micrococcus sp, and bacillus sp. were occasionally isolated. Occasionally a coliform micrococcus and/or bacillus sp. would be isolated from kidney tissues. From the tongue and throat swabs, bacillus sp, pseudomonas sp, micrococcus sp, alpha strep and coliforms were commonly isolated and proteus was occasionally isolated. In one case of ocular discharge, a Staphylococcus and a coliform were isolated. Otherwise all other organs were negative for bacteria.

Nine of the twenty eight (28) animals had slight to heavy infestations of spargana, and one of five (5) carcasses examined had 3rd stage larva of Gnastostoma species. Results of necropsy examinations are summarized in Table IV.

Discussion: The ease with which the tree shrews adapted to dog food, a cheap, readily available and easy to handle diet simplified this phase of the investigation. After the initial phase of adaptation to the environment, during which time the condition of some animals progressively deteriorates, the surviving animals appear to thrive on this diet. Most of the animals purchased so far are sub-adults or young adults. In the first mating season 5 of 27 females held in captivity for more than 5 months have become pregnant. Since these animals are reported to be reluctant to breed in captivity, this is a very encouraging rate of conception. Further, the only one of these five to have given birth so far, is nursing the baby very well and is presenting encouraging signs of overcoming a second difficult phase - rearing the young in captivity. The biological values obtained on the

sacrificed animals are too few to make strong comments about. Not enough numbers are available to determine if there is or not a sex difference in the hematology and blood chemistries values.

In Table III some interesting values are presented. The only pregnant female killed had an extremely high alpha₂ globulin (1.1 mg%) value compared with 0.2 to 0.4 for all other female tree shrews examined. Also two female shrews had split beta globulin peaks and two male shrews had no detectable alpha₂ globulins. Attempts to correlate these findings with an abnormal state have not been done. The bacteriology results are not remarkable and can be considered as normal bacterial flora. The high incidence of *Spargana* is surprising since shrews are reported to be fructivorous and insectivorous. This finding suggests the inclusion of frogs, toads, etc., as part of their native diet.

Summary: Tree shrews (*Tupaia glis*) have been shown to survive on commercial dry dog meal when housed in various type cages. Hematology, biochemistry, bacteriology, parasitology and necropsy results are presented on sacrificed animals. This study is continuing.

Table I
BLOOD CHEMISTRY

| | Average | FEMALE Range | Number | Average | MALE Range | Number | Average | TOTAL Range | Number |
|---------------------------------|---------|-----------------|--------|---------|---------------|--------|---------|----------------|--------|
| Thymol (Thymol Units) | 1.7 | - | 1 | 1.2 | 0.7-2.0 | 3 | 1.3 | 0.2-2.0 | 4 |
| Cholesterol (mg%) | 89 | 55-193 | 5 | 92 | 52-132 | 7 | 91 | 52-193 | 12 |
| Urea N ₂ (mg%) | 34.2 | 13.7-84.4 | 11 | 37.5 | 21.2-87.6 | 14 | 36.1 | 13.7-87.6 | 25 |
| Protein (gm%) | 5.9 | 3.5-7.4 | 10 | 5.8 | 4.3-6.8 | 12 | 5.8 | 3.5-7.4 | 22 |
| Alkaline Phosphatase (Sigma) | 3.8 | 2.9-5.3 | 6 | 4.0 | 1.1-7.5 | 5 | 3.9 | 1.1-7.5 | 11 |
| SGOT (S.F. Units) | 153 | 94-237 | 5 | 180 | 103-250 | 6 | 168 | 94-250 | 11 |
| SGPT (S.F. Units) | 19 | 11-28 | 5 | 39 | 19-89 | 5 | 29 | 11-89 | 10 |
| Bilirubin Direct (mg%) | 0.07 | 0.0-0.1 | 7 | 0.12 | 0.05-0.2 | 9 | 0.10 | 0.0-0.2 | 16 |
| Total | 0.19 | 0.0-0.3 | 7 | 0.3 | 0.2-0.5 | 9 | 0.25 | 0.0-0.5 | 16 |
| CO ₂ (mM/L) | 14.5 | 10.0-22.0 | 9 | 16.3 | 8.6-21.9 | 10 | 15.5 | 8.6-22.0 | 19 |
| Na ⁺ (Meq/L) | 163 | 155-178 | 7 | 154 | 117-178 | 8 | 158 | 117-178 | 15 |
| K ⁺ (Meq/L) | 4.5 | 3.1-5.8 | 7 | 4.6 | 3.9-6.7 | 8 | 4.6 | 3.1-6.7 | 15 |
| Cl ⁻ (Meq/L) | 118.2 | 110.8-125.0 | 7 | 116.3 | 106.4-126.9 | 7 | 117.3 | 106.4-126.9 | 14 |

Table II
HEMATOLOGY

| | FEMALE | | | MALE | | | TOTAL | | |
|-------------------------|---------|----------|--------|---------|-----------|--------|---------|-----------|--------|
| | Average | Range | Number | Average | Range | Number | Average | Range | Number |
| Hct (%) | 38.2 | 33-44 | 9 | 43.8 | 29-57 | 13 | 41.5 | 29-57 | 22 |
| Hgb (gm%) | 11.7 | 8.9-13.7 | 9 | 13.5 | 8.6-17.6 | 13 | 12.8 | 8.6-17.6 | 22 |
| WBC (x1000) | 2.71 | 0.7-7.2 | 9 | 2.52 | 0.8-7.2 | 13 | 2.60 | 0.7-7.2 | 22 |
| RBC (x10 ⁶) | 5.79 | 4.0-7.07 | 9 | 6.81 | 3.58-8.89 | 13 | 6.39 | 3.58-8.89 | 22 |
| N (%) | 72.6 | 54-90 | 9 | 68.8 | 36-90 | 13 | 70.4 | 36-90 | 22 |
| L (%) | 20.3 | 0-42 | 9 | 23.3 | 2-62 | 13 | 22.1 | 0-62 | 22 |
| B (%) | 2.1 | 0-5 | 9 | 0.85 | 0-4 | 13 | 1.36 | 0-5 | 22 |
| E (%) | 3.89 | 0-16 | 9 | 4.62 | 0-14 | 13 | 4.32 | 0-16 | 22 |
| Baso (%) | 1.1 | 0-4 | 9 | 1.15 | 0-4 | 13 | 1.14 | 0-4 | 22 |
| M (%) | 1.1 | 0-6 | 9 | 1.23 | 0-5 | 13 | 1.18 | 0-6 | 22 |

Table III
ELECTROPHORESIS - SERUM PROTEIN VALUES

| | FEMALE | | | MALE | | | TOTAL | | |
|--------------------------|---------|---------|--------|---------|-----------|--------|---------|---------|--------|
| | Average | Range | Number | Average | Range | Number | Average | Range | Number |
| Total Globulin (gm%) | 4.16 | 2.8-5.6 | 9 | 3.71 | 2.7-5.1 | 9 | 3.93 | 2.7-5.6 | 18 |
| Alpha ₁ (gm%) | 0.35 | 0.3-0.4 | 9 | 0.33 | 0.2-0.5 | 9 | 0.34 | 0.2-0.5 | 18 |
| Alpha ₂ (gm%) | 0.29 * | 0.2-0.4 | 8 | 0.3 | 0.0-0.5** | 9 | 0.29 | 0.0-0.5 | 17 |
| Beta (gm%) | 1.82 | 0.8-3.1 | 9 | 1.74 | 1.1-2.9 | 9 | 1.78 | 0.8-3.1 | 18 |
| Gamma (gm%) | 1.6 | 0.8-2.1 | 9 | 1.3 | 0.3-1.8 | 9 | 1.4 | 0.3-2.1 | 18 |
| Albumin (gm%) | 1.94 | 1.1-3.0 | 9 | 2.13 | 1.1-3.6 | 9 | 2.04 | 1.1-3.6 | 18 |
| A/G Ratio | 0.5 | 0.3-1.0 | 9 | 0.6 | 0.2-1.3 | 9 | 0.6 | 0.2-1.3 | 18 |

* 1 pregnant female with 1.1 alpha₂ value
 ** 2 males with liver damage 0 alpha₂
 + 2 females had a split peak

Table IV

SUMMARY OF NECROPSY FINDINGS BY ORGAN SYSTEMS

1. SKIN

- a. 2 animals had numerous bites from ectoparasites.
- b. 1 animal had yeast-like fungal infection of the skin.
- c. 9 animals had spargana in subcutaneous tissues, 4 with heavy infection; the worms were most frequently found in the region of the groins or lower abdomen, followed by the axillae, flanks and instrascapular area in that order.

2. SKELETAL MUSCLE

- a. Sarcosporidia were found in 1 animal.
- b. Larvae of *Gnathostoma* species, third stage were found in 1 animal.
(Dr. Svasti Daengsvang).

3. EYES

- a. 1 animal had a foreign body in the eye.
- b. 1 animal had inflammation of the right cornea.
- c. 1 animal had whitish sticky discharge from both eyes; no inflammation seen on eyeballs on microscopy.

4. SALIVARY GLANDS

1 animal had enlargement of the salivary glands with ductal hyperplasia.

5. HEART

- a. 1 animal, which was found dead, had large hemorrhages in the heart; 10 other animals had small hemorrhages probably due to cardiac puncture for blood.
- b. 1 animal had dextro-cardia.
- c. 2 animals had striking numbers of mast-like cells in the myocardium.

6. LUNGS

- a. 3 animals had granulomata containing nematode larvae in various stages of development (identified as *Toxocara sp.* by Dr. Crooks).
- b. 6 animals had lymphoid nodules or patches near bronchi, very marked in 1 animal.
- c. 16 animals had congestion of blood vessels in 2 animals there were hemorrhages (1 which was found dead, also had hemorrhagic edema of paratracheal connective tissue).

Table IV (Continued)

7. LIVER

- a. 7 animals had fatty change of the liver cells, 4 slight only, 1 quite marked.
- b. 3 animals had focal necrosis, not marked.
- c. 6 animals had inflammation of the portal tracts, 1 with slight fibrosis.
- d. 4 animals had congestion of central veins and/or sinusoids, (1 was found dead).
- e. In 7 animals, the liver cells, particularly in centrolobular areas, were markedly vacuolated.
- f. 1 animal had a cyst containing a parasite.
- g. In 2 animals the liver was autolysed (found dead).

8. SPLEEN

- a. 4 animals had congestion of the spleen; 2 of these also had hypertrophy of the follicles, 1 with very large germinal centers (found dead); 1 of the animals had thick capsule and trabeculae.
- b. 2 animals had neutrophiles in the red pulp.

9. PANCREAS

- a. 1 animal (TS #4) had a cyst containing a parasite with eggs.

10. GASTROINTESTINAL TRACT

- a. 6 animals had parasites in the submucosa of the stomach, 5 had parasites in the intestines, (2 had parasites in both).
- b. 4 animals had eosinophiles in considerable numbers in the mucosa, but no parasites were found.
- c. 2 animals had lymphoid patches in the stomach and 1 in the intestine.
- d. 1 animal (TS #6 found dead), had congested and distended intestine sm, markedly congested omentum, and greyish yellow exudate in the peritoneal cavity.

11. URINARY TRACT

- a. 1 animal had (TS #4) chronic pyelonephritis.
- b. 4 animals had some casts in the tubules.
- c. 6 animals had lymphoid nodules or patches near the renal pelvis.
- d. 1 animal had generalized congestion 1 had slightly congested glomeruli.
- e. 1 animal (TS #2, with worm larvae in the lungs) had a granuloma with dee developing nematode embryo on the serosal surface of the bladder wall.

Table IV (Continued)

12. REPRODUCTIVE ORGANS

No abnormal changes were found in the males.

1 female was pregnant.

2 females were lactating.

4 females had neutrophils in the uterine lumen.

2 females had congestion of the uterus and thick endometrium.