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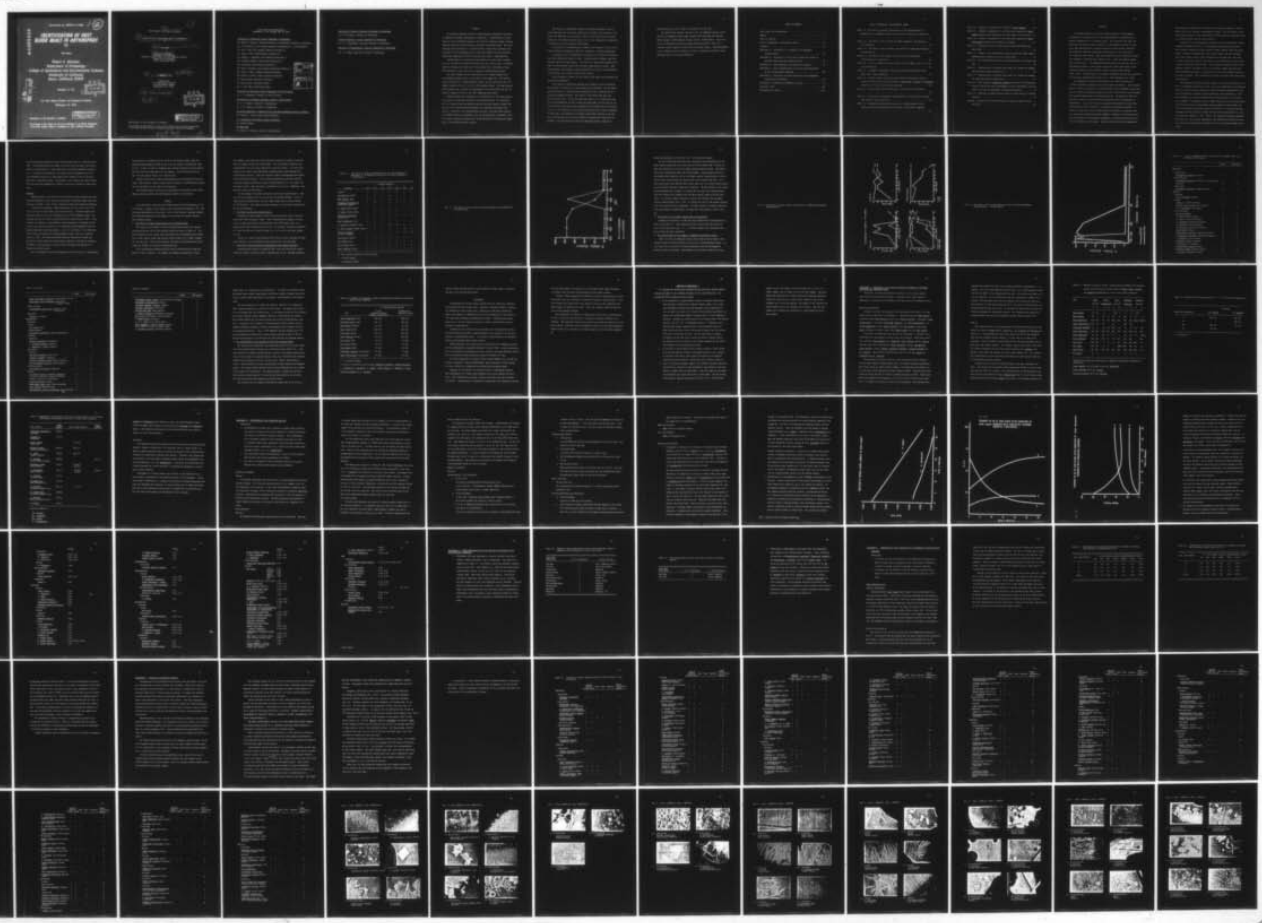

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IDENTIFICATION OF HOST BLOOD MEALS IN ARTHROPODS (U)

Final Report

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November 25, 1977

U.S. Army Medical Research and Development Command
Washington, D.C. 20314

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Summary

An accurate knowledge of hosts of hematophagous arthropods is essential in studies of diseases transmitted by arthropods. Identification of blood meals of these arthropods requires a test which is sensitive enough to detect digested blood and specific enough to identify the various hosts. The study presently being reported on is concerned with a method which involves crystallization of the hemoglobin (Hb) in the blood samples from the arthropod midgut and comparing the crystal structure with that of known material.

Crystallization of vertebrate blood samples were more easily attained with the relatively insoluble Hb than with the more soluble ones. Techniques aimed at reducing the solubility of more soluble Hb's, buffering at human Hb's isoelectric point, were used to improve crystallization success.

The final reagents for Hb crystallization of a midgut sample from a blood engorged mosquito was to treat half of the sample with .035M ammonia oxalate, pH 6.86, in a 0.025M phosphate buffer, and the other half with .07M ammonia oxalate, pH 6.15 $\pm$ .02 in a 1.5M phosphate buffer. The high molarity buffer reagent was to ensure crystallization of cow blood meals and the low molarity reagent for most of the other mammalian blood meals.

Further studies were conducted on the variability of the crystal growth with respect to different mosquitoes and vertebrate hosts. Hb solubility and to a lesser extent, the mosquito species involved, appear to govern the success of the crystallization technique for individual vertebrate host species. Therefore, the crystallization success of any blood meal containing soluble Hb may be considerably less in some mosquitoes; conversely, with blood meals containing insoluble Hb, little difficulty is encountered regardless of the mosquito species involved.

The results of preliminary isoelectric focusing experiment with cow blood indicated that cow Hb was altered quite rapidly in the process of the blood meal digestion in the mosquito, and it is this rapid loss in concentration of the soluble Hb which plays a part in the reduction of crystallization success with the presently used reagent.

On the basis of electrophorectic studies, there appears to be no correlation between electrophoretic mobility, solubility or crystal morphology.

No changes in the electrophoretic properties of cow blood were observed even after prolonged storage (1 year). Crystallization, however, was less reliable than with fresh samples. The results of identifying multiple blood meals by the Hb method varied with different combinations of hosts. Some blood mixtures were negative; others produced one or both of the individual crystal forms; still others formed hybrid crystals.

Limited samples of known tick and bedbug blood meals were processed and identified successfully.

The accuracy of identifying unknown blood samples by the Hb technique was assessed by conducting (1) blind tests in the laboratory, and (2) identifying blood meals of field-collected mosquitoes by the precipitin and Hb tests and comparing results. The results of the blind tests were low (64 and 56 per cent). Scores for some blood meals (i.e., horse) were high (24 of 24) irrespective of time; others were high (cow, 8 of 8) only for the first 6 hours; still others were consistently low irrespective of time (man, 4 of 17). The comparison of the Hb with precipitin test is still incomplete. At this stage, the proportion of mosquito blood meals reacting to any host with the Hb method (51.6 per cent of 860) is significantly lower than the results of the precipitin test when the mammalian-negative category is

excluded (97.8 per cent of 1408) or included (77.0 per cent).

Hb crystals were induced from 93% of the 170 mammalian species tested. 89.5% of the mammals tested formed crystals with the normal low molarity buffer reagent (0.035M ammonia oxalate, pH 6.8, 0.025M buffer). Only 46.7% of the 45 different bird species tested produced cyrstals; three of 13 reptiles tested formed Hb crystals with the normal reagent. Photomicrographs of the crystals of most vertebrates tested is catalogued according to host phylogeny and/or Hb crystal morphology.

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Foreword

A detailed knowledge of the blood feeding patterns of hematophagous arthropods is an essential part of any epidemiological investigation of a disease transmitted by an arthropod vector. The precipitin test (Bull and King 1923) has long been and continues to be the method of choice for host blood meal identification in mosquito and other blood sucking arthropods. The method has undergone considerable modifications as the "ring test", the "capillary tube method", "agar gel diffusion" and the "microplate method" (see summaries by Boreham 1975; Tempelis 1975). Other serological methods have also been used. The hemaglutination-inhibition test has been used as a supplementary method to gain greater specificity (Weitz 1956; Murray 1970; Tempelis and Rodrick 1972; Tempelis, Reeves and Nelson 1976; Boorman et al. 1977). A method based on flourescent antibodies has also been described for blood meal identification (Gentry et al. 1967; McKinney et al. 1972).

The hemoglobin crystallization method for host meal identification utilizes a different principle and is both simple and specific. The technique appears to have the potential of being developed into a dependable operational tool for medical entomology purposes, and if so, it would offer many advantages over existing methodologies previously mentioned. The simplicity makes its possible use ideal in situations where serological work is impractical or inaccessible. It may also be used to separate blood samples of some closely related forms which are difficult to separate by conventional methods. It would be a very useful tool in areas where the diversity of potential hosts may over-extend available blood meal samples. Contrary to the multiple testing system required in serological techniques, this method requires only one test to identify the host animal. Finally, soon after blood is ingested by

some mosquitoes, plasma may be excreted which causes the density of the red blood cells in the stomach to approximately double. For this reason a technique utilizing the cellular constituents of blood may be more desirable than a test which depends upon the serum proteins.

Studies on the crystallographic properties of Hb in blood meals of hematophagous invertebrates have been investigated intermittently by several early workers including Stirling and Brito (1882), Amantea (1926) and Bioca (1950). Working with mosquitoes, ticks and/or leeches and six vertebrate hosts with varying success, these investigators concluded that "the study of these crystals may lead in some cases to the identification of the animal species to which the blood belongs". More recently, Washino and Else (1972) produced crystals from whole blood of 11 different animals and from blood meals extracted from midgut of mosquitoes 24 hours after feeding of eight of the same 11 animals. This constituted the basis from which the present study was developed.

The developing technique presently allows the possible identification of 94% of the mammals studied and as such, can be most applicable in epidemologic study of such vector-borne diseases as Venezuelan Equine Encephalitis, California Encephalitis, Western Equine Encephalitis and Leishmaniasis. Since small mammals, particularly rodents and/or leporids appear to be involved in the endemic or tangentinal cycle of these diseases, the Hb crystallization would be useful since crystallization patterns among 15 rodent genera and often even between species were found to be distinct. In certain diseases, instances involving specific primary host that have insoluble Hb (i.e., dog and heartworm; equines in VEE, WEE), this technique would have immediate practical use. With further development, the technique can become an essential part of any investigation procedure for all vector-borne disease situations.

EXPERIMENT 1: FORMULATION OF A REAGENT(S) FOR HEMOGLOBIN CRYSTALLIZATION.

Objectives:

1. To study specific factors which affect hemoglobin crystallization;
2. To formulate the optimum crystallizing agent based on the results of (1).

Factors which have been shown to influence protein crystallization include temperature, pH, the nature of the salt ion, and protein composition (Czok & Bucher, 1960; Dixon & Webb, 1961; Zeppezauer, 1971). The factors manipulated in this study were: 1) salt concentration, 2) pH and 3) the effect of ligand (cyanide) concentration. The success of the various test reagents, in producing crystals of characteristic form from Hb lysates of various hosts, determined the formulation of the optimum crystallizing agent. The formulated reagent was further tested against: 1) hemolysates from 100 mammalian species, and 2) mosquito blood meals from identified hosts.

Materials and Methods

Whole blood was obtained by venipuncture, collected in sodium heparin and preserved with sodium azide. More than one sample of each species was tested when available. The blood samples were centrifuged at 3,000 rpm for ten minutes and the plasma removed. The remaining erythrocytes were washed three times with 0.15M NaCl and then lysed by the addition of an equal volume of distilled water. The resulting lysate was centrifuged at 3,000 rpm for 10 minutes, the supernatant removed and stored at $-20^{\circ}C$.

Hb crystal preparation from hemolysates

Equal volumes of hemolysate and test reagent were mixed together. One drop of this solution was placed on a clean glass slide and left until a

definite dried ring had formed around the periphery. A coverslip was then placed on the drop and the developing crystals were observed with a compound microscope. The slides were examined immediately, after four hours, 24 hours and 4 days. Two hours after covering, the edges of the coverslip were sealed with permount® (Fisher Scientific Co.) and held at 4°C. Hb lysates were used for all experiments except the blood meal identification (Experiment F), and each treatment was replicated 10 times.

Blood meal preparation

The following mosquito species were collected as larvae and adults from the Sacramento Valley and the Sierra Nevada in California: Anopheles freeborni, Aedes increpitus, Ae. cataphylla, Ae. melanimon, Culex pipiens, Cx. tarsalis, Cx. peus, Culiseta inornata and Cs. incidens. Aedes aegypti (Liverpool strain) and Cx. tarsalis (Owens Valley strain) from established laboratory cultures were also used. Only mosquitoes collected from the field as larvae or pupae, were fed on test animals. To obtain blood meals, 20 female mosquitoes were placed in a small feeding cage, which was taped to the ear (in the case of the larger domestic animals) and left for 15 minutes. Smaller animals were restrained in a mesh bag and placed on top of the mosquito cage. Feedings were performed in the early morning or evening. The engorged mosquitoes were kept at 22°C for 6 and 24 hours after blood ingestion. The midgut was then removed and stored at -70°C in gelatin capsules within a glass vial to be processed later. Field collected adults were fed on fresh heparinised blood through a mouse skin membrane.

Hb crystal preparation from mosquito blood meals

Each blood meal was placed in a microtiter well and ten microliters of the test reagent added. The midgut was macerated with a small glass pestle

and the resulting suspension removed from the micro-well by a 32mm capillary tube. The capillary tube was sealed at one end with clay sealer and centrifuged for five minutes in a micro-hematocrit centrifuge (Drummond Scientific Co.). Following centrifugation, the sealed end with sediment was cut off, the supernatant placed on a clean glass slide, allowed to dry at the periphery and a coverslip applied. The margin of the coverslip was sealed within the first hour and examined for crystals as with the erythrocyte lysate solution.

Reagents

Ammonium oxalate, the salt used in the initial studies (Washino and Else, 1972) was examined in more detail and preliminary comparisons made with other reagents including: ethyl alcohol, ammonium sulphate and potassium phosphate.

In the first experiment, oxalate molarities of 0.21, 0.14, 0.11, 0.07, 0.04, 0.02 and 0.01 were made up in distilled water. Buffered oxalate solutions were used for all later experiments. The 0.025M phosphate buffer was prepared from the two stock solutions which were kept frozen at -20°C. Stock solution I consisted of 0.2M monobasic sodium phosphate (2.78 gm/100 ml distilled water) and stock solution II, 0.2M dibasic sodium phosphate (5.36 gm $Na_2HOP_4.7H_2O$/100 ml distilled water). 51ml of I and 40ml of II in 100ml distilled water gave 200ml 0.1M sodium phosphate buffer at pH 6.8. This was stored at -20°C. The required oxalate solution, buffered at 0.025M, was prepared by simply adding the correct molar quantity of oxalate for dilution of 100ml of distilled water, to 25ml of the 0.1M buffer and making up to 100ml with distilled water.

The pI (isoelectric point) for human and cow are 6.8 and 6.5, respectively.

Crystallization of human Hb and of the Hb of two bovine breeds, Angus and Charolais was examined at these two pH's over an oxalate concentration range of 0.02 - 0.21M, in order to establish the optimum conditions for each species and effect the best compromise for the reagent. Horse and pig Hb were run with the same oxalate range, but at pH 6.8 only.

Oxalate crystals were easily distinguished from the different Hb crystal forms. When present, oxalate crystals were colorless, of characteristic shapes and occurred mainly at the edge of the coverslip.

The cyanide reagent was the given concentration of cyanide in the 0.035M ammonium oxalate solution (0.025M phosphate buffer) at the test pH.

RESULTS

Test reliability increased after plasma removal and centrifugation of the blood sample. Renewal of the reagents every two weeks and storage at 4^{o}C. also increased repeatability of the tests. Use of ethyl alcohol, ammonium sulphate and potassium phosphate did not improve or even match the results obtained with ammonium oxalate.

A. The effect of oxalate concentration on Hb crystallization

The results of the range of oxalate concentrations tested that induced crystallization for the 15 host species studied are given in Table 1-1. The more insoluble Hb (e.g., goat, horse and maned wolf) crystallized more rapidly over a wider oxalate range than the more soluble forms (e.g., human, chimpanzee, cow and pig). This was particularly true when the holding time of blood slides was limited to a 24-hour incubation period.

Salt concentration modified both the form of crystals and the time and extent of their formation. For example, the immediate production of long,

fine needles, from which the large distinctive polyclinic plates of horse Hb form, no longer occurred at 0.02M oxalate. The long needles formed at this concentration, but more slowly and did not grow into plates. Cow and pig Hb crystals were almost indistinguishable morphologically, when produced with 0.02M ammonium oxalate. They were, however, easily distinguished when formed with 0.035M oxalate reagent. Lower oxalate concentrations (0.02M) required increased incubation periods to induce crystallization (e.g., cat, goat, cow and horse), while other Hb did not crystallize at all (e.g., chimpanzee, man, walleroo, pinon and bush mouse).

It would appear that higher molarities favored Hb crystallization. This was true with primates, but not so with pig and clouded leopard. Of the 15 animal species examined here, only the pigmy chimp failed to form crystals at 0.035M oxalate. This oxalate molarity appeared to be the most versatile for Hb crystal production.

B. The effect of pH on Hb crystallization

Proteins are least soluble at their isoelectric points (pI) at low salt molarities (Czok and Bucher, 1960). This relationship was evident in effect of pH on human and bovine Hb crystallization at 0.035M oxalate (Fig. 1-1). Human blood formed crystals only at pI 6.8. In contrast, cow would crystallize at a lower pH and over a wider pH range (i.e., 6.6 to 6.0) with best results at 6.15.

pH was not a major crystallization factor with the more insoluble Hb (dog, horse and goat), but influenced the more soluble Hb of cow and human.

C. The effect of pH and oxalate concentration on Hb crystal formation

Crystallization success of human Hb (Fig. 1-2a) over its active oxalate range was severely reduced at pH 6.5 compared with pH 6.8. Maximum crystalli-

Table 1-1. The effect of oxalate concentration on the crystallization of hemoglobin of 15 mammalian species over 24 hours incubation. (5 replicates).

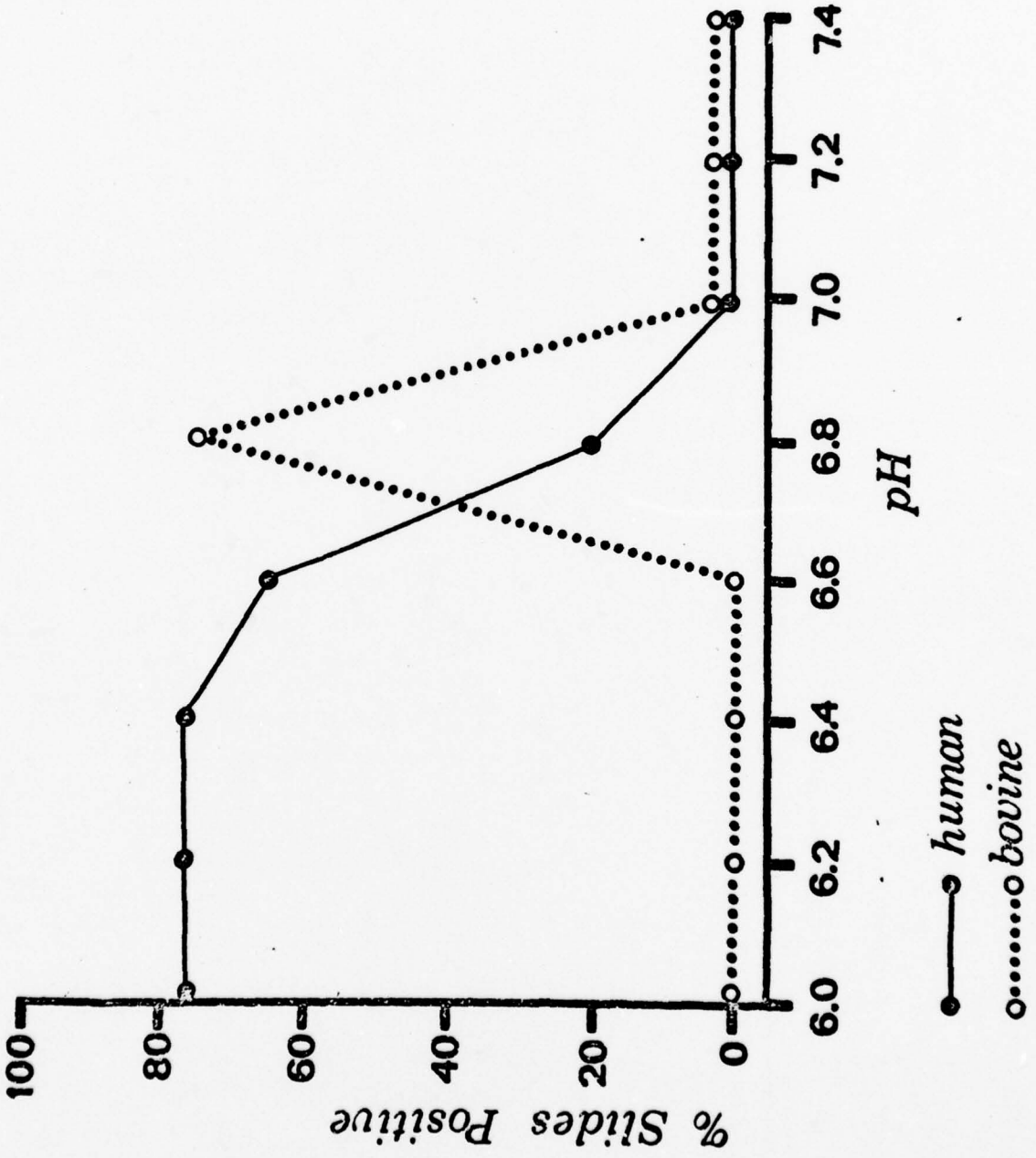
	Oxalate Molarity						
Species	.21	.14	.11	.07	.04	.02	.01
Walleroo	+	+	+	+	+	-	-
Pan paniscus (chimp)	+	+	+	++	-	-	-
Homo sapiens (man)	+	+	+	++	+	-	-
Peromyscus californicus (california mouse)	+	++	+	+	+	+	+
P. truei (pinon mouse)	-	+	+	+	+	-	-
P. boyeli (brush mouse)	-	+	+	+	+	-	-
Chrysocyon brachyurus (maned wolf)	+	+	+	+	+	+	+
Felis domesticus (cat)	+	+	+	+	+	-	-
F. concolor (mountain lion)	+	+	+	++	++	+	+
F. tigris tigris (bengal tiger)	+	+	+	+	+	++	+
Neofelis nebulosa (clouded leopard)	-	-	-	-	+	++	++
Bos taurus (bovine)	+	+	+	+	+	-	-
Sus scrofa (pig)	-	-	-	-	+	+	+
Capra hircus (goat)	+	+	+	+	+	+	+
Equus caballus (horse)	+	+	+	+	+	+	+

++ best crystal formation for that species.

\+ crystals formed.

\- no crystals formed.

Fig. 1-1 The effect of pH on bovine and human hemoglobin crystallization. (10 replicates).


% Slides Positive
100
80
60
40
20
0
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
pH
human
bovine

zation was obtained at pH 6.8 with 0.04 - 0.06 molarity oxalate.

The pH 6.5 buffered solutions were consistently more successful over the whole oxalate range than pH 6.8 with the two bovine breeds (Fig. 1-2b and 1c). This was particularly true at the lowest oxalate molarity tested. Two differences were noted between these two cattle breeds. Crystallization was more predictable with Charolais, and at the higher oxalate concentrations, a second crystal form (thin plates) was found as well as the normal rods (Fig. 1-2c). These plates have been observed with jersey cows, but in no other breeds studied by us (hereford, guernsey, short horn, holstein). The Hb crystals of all cattle breeds examined had a tendency to broaden with the lower salt concentrations. Horse Hb crystals were produced over the whole oxalate range at pH 6.8 (Fig. 1-2d). At this pH crystal production of pig Hb was reduced with increased oxalate concentrations (Fig. 1-2d). It appears that pH 6.8 and oxalate molarity 0.04 is the most efficient compromise. A slight increase in oxalate concentration would benefit crystallization of human Hb at the expense of that of the pig.

D. The effect of the cyanate ligand on Hb crystallization

Cyanides at molarities of 0.062 and above inhibited Hb crystallization in horse blood (Fig.1-3). This inhibitory effect was not seen with bovine Hb until 0.25M cyanide was used. It did increase slightly the consistency with which bovine Hb would crystallize.

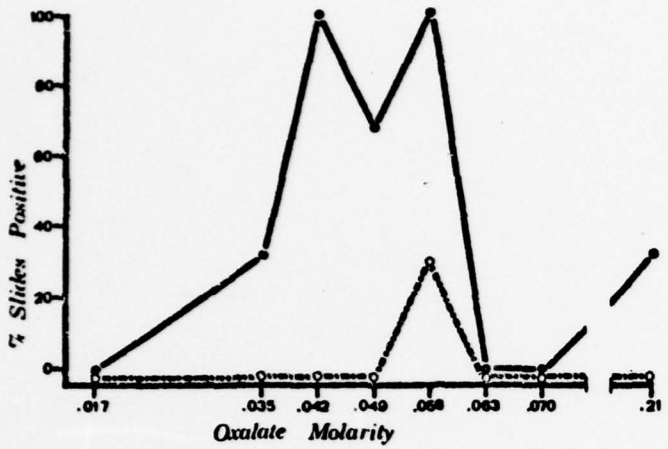
E. The effectiveness of the reagent on mammalian erythrocyte lysates

Table 1-2 lists the mammalian species tested with either 0.035M or 0.07M ammonium oxalate solution buffered to pH 6.8 by a 0.025M phosphate buffer. In this paper only the success of crystal production from the 100 mammalian species tested is discussed. Descriptions of the crystal forms will be pub-

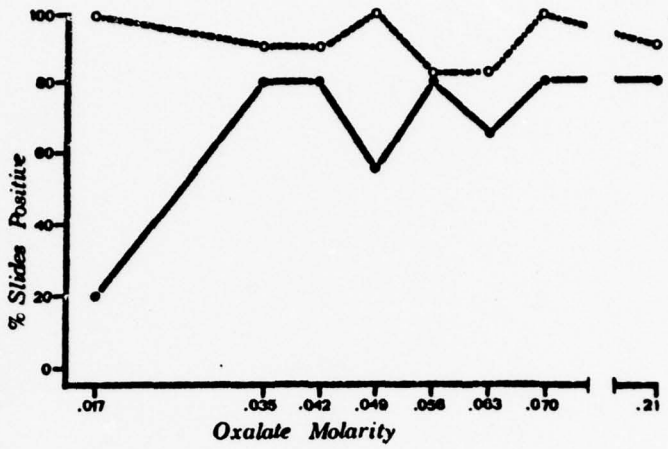
Fig.1-2 The effect of pH and oxalate concentration on mammalian hemoglobin crystallization.

(10 replicates) ------ *pH 6.5*; —— *pH 6.8*

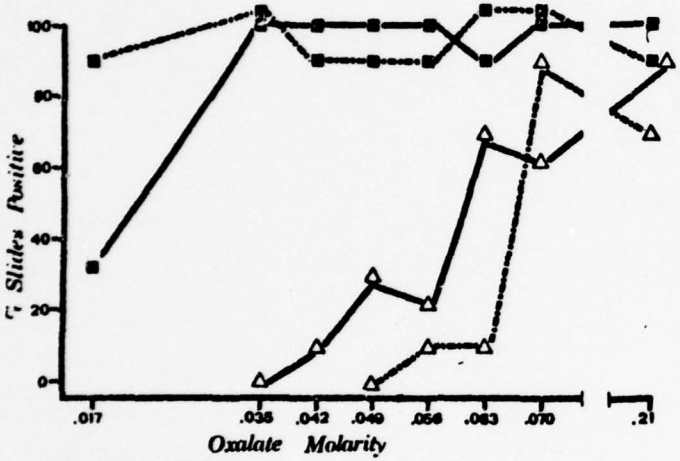
a) Human Hb



b) Angus Hb



c) Charolais Hb



d) Horse and pig Hb

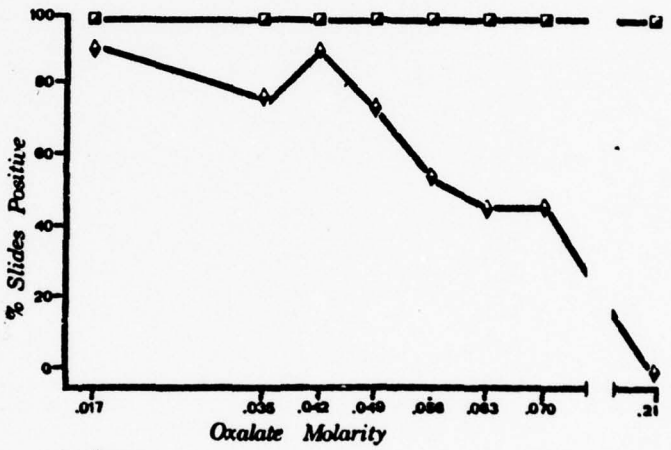


Fig. 1-3 The effect of the cyanate ligand on bovine and horse hemoglobin crystallization. (9 replicates).

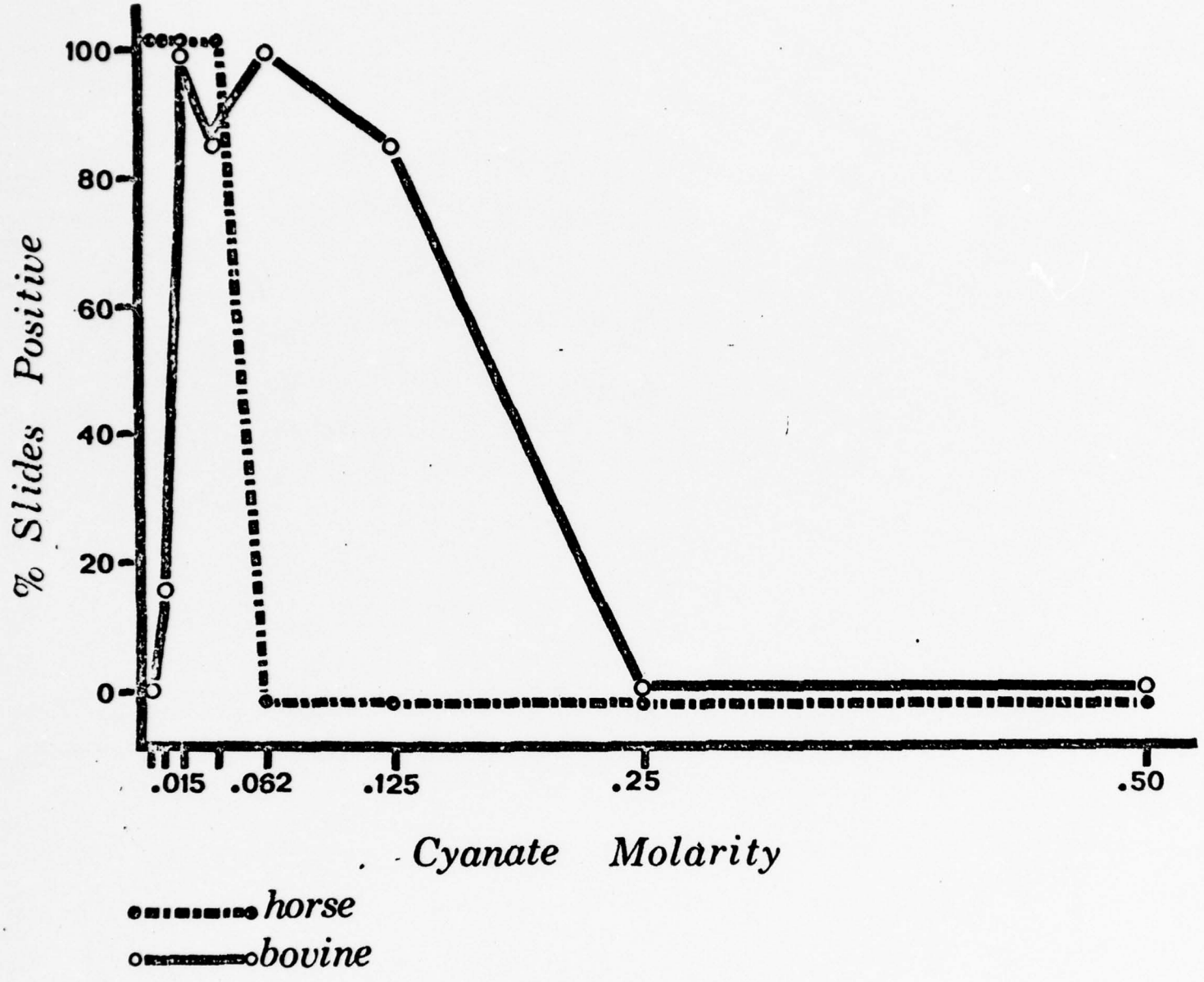

100
80
60
40
20
0
% Slides Positive
.015
.062
.125
.25
.50
Cyanate Molarity
horse
bovine

Table 1-2. A list of mammalian species tested with the reagent, pH 6.8, at 0.035M and 0.07M oxalate.

	0.035M	.07M oxalate
MARSUPIALS		
Didelphoidea		
Didelphis marsupialis (opossum)	+	+
Marcropodidae		
Dendrolagos matsechi (Matschie's Tree Kangaroo)	+	+
Walleroo	+	+
Dasyuroidea		
Sarcophilus harrisii (Tasmanian devil)	+	+
PRIMATES		
Lemuridae		
Lemur variegatus (Lemur)	-	-
Cebidae		
Ateles sp. (spider monkey)	+	+
Cacajao calvus calvus (white uakari)	+	+
C. c. rubicundis (red uakari)	+	+
Cercopithecidae		
Macaca arctoides	+	+
M. rhesus (Rhesus monkey)	+	+
M. iris (crab-eating macaque)	+	+
M. cynomolgus (cynomolgus monkey)	+	+
M. sylvanus (Barbary macaque)	+	+
M. nemistrina (pig-tailed macaque)	+	+
Papio anibis (baboon)	+	+
Comopithecus hamadryas (Hamadryas baboon)	+	+
Cercopithecus nictitans (spot-nosed guenon)	+	+
C. aethiops (vervet monkey)	+	+
Mandrillus sphinx (mandrill)	-	-
Cercocebus sp. (mangabey)	-	-
Theropithecus gelada (gelada)	-	-
Presbytis johnii (Nilgiri langur)	+	+
P. entellus (Hanuman's langur)	+	+

Table 1-2 (cont'd)

	0.035M	0.7M oxalate
Nasalis larvatus orientalis (Proboscis monkey)	+	+
Pongidae		
Pan paniscus (Pigmy chimpanzee)	-	+
Pan troglodytes verus (western chimpanzee)	+	+
Hominidae		
Homo sapiens (man)	+	+
RODENTIA		
Sciuromorpha		
Sciuridae		
Sciurus griseus (gray squirrel)	+	+
Eutamias sp. (chipmunk)	+	+
Spermophilus beecheyi (California ground squirrel)	+	+
Myomorpha		
Heteromyidae		
Dipodomys sp. (kangaroo rat)	+	+
Cricetidae		
Peromyscus truei (Pinon mouse)	+	+
P. californicus (California mouse)	+	+
P. maniculatus (deer mouse)	+	+
P. boylei (brush mouse)	+	+
Neotoma lepida (desert wood rat)	+	+
N. cinerea (bushytail wood rat)	+	+
Sigmodon hispidius (cotton rat)	+	+
Microtus longicaudis (Vole)	+	+
Reithrodontomys megalotis (harvest mouse)	+	+
Muridae		
Mus musculus (house mouse)	+	+
Rattus rattus (roof rat)	+	+
R. norvegicus (Norway rat)	+	+
Hystricomorpha		
Erethizoa dorsatum (porcupine)	+	+
Caviidae		
Cavia porcellus (guinea pig)	+	+
Lagomorpha		
Lepus californicus (jackrabbit)	+	+

Table 1-2 (cont'd)

	0.035M	.07M oxalate
Sylvilagus auduboni (desert cottontail)	+	+
CARNIVORA		
Canidae		
Canis latrans (coyote)	+	+
C. familiaris (dog)	+	+
C. lupes (wolf)	+	+
C. aureus (golden jackal)	+	+
Chrysocyon brachyurus (maned wolf)	+	+
Urocyon cinereoargentus californicus (California gray fox)	+	+
Procyonidae		
Procyon lotor (racoon)	-	-
Mustelidae		
Mephitus mephitus (striped skunk)	+	+
Pinnipedia		
Arctocephalus sp. (fur seal)	+	+
Mirounga angustirostris (Northern elephant seal)	+	+
Felidae		
Felis domesticus (cat)	+	+
F. concolor (mountain lion)	+	+
Panthera leo (African lion)	+	+
P. tigris tigris (Bengal tiger)	+	+
P. tigris altaica (Siberian tiger)	+	+
P. onca (jaguar)	+	+
P. pardus japonensis (north Chinese leopard)	+	+
P. pardus delacouri (Indo-Chinese leopard)	+	+
Neofelis nebulosa (clouded leopard)	+	-
Acinonyx jubatus (cheetah)	+	+
PAENUNUGULATA		
Hyracoidae		
Procavia capensis capensis (South African rock hyrax)	+	+
PERISSODACTYLA		
Equidae		
Equus caballus (horse)	+	+

Table 1-2 (cont'd)

	0.035M	.07M oxalate
Equus przewalski przewalski (Przewalski's horse)	+	+
Hippotigris zebra hartmannae (Hartmann's mountain zebra)	+	+
Rhinocerotidea		
Ceratotherium simum simum (southern white rhinoceros)	+	+
Tapiridae		
Tapirus sp. (Tapir)	+	+
ARTIODACTYLA		
Suiformes		
Suidae		
Sus scrofa (pig)	+	+
Hippopotamidae		
Choeropsis liberiensis (pigmy hippopotamus)	+	+
Tylopoda		
Camelidae		
Camelus dromedaraus (dromedary)	+	+
L. guanacoe guanacoe (guanaco)	+	+
L. guanacoe f. glama (guanaco)	+	+
Ruminantia		
Cervidae		
Odocoileus hemionus (mule deer)	+	+
Rangifer tarandus (reindeer)	-	-
Muntiacus reevesi reevesi (Reeves muntjac)	+	+
Cervus elaphus sibiricus (Altai wapiti)	+	+
Antilocapriadae		
Antilocapra americana (pronghorn)	+	+
Bovidae		
Bos taurus (jersey, hereford, guernsey, shorthorn, Charolais, angus & holstein)	+	+
Beefalo (13/16 cow, 3/16 bison)	+	+
Bison bison bison (bison)	+	+
Hippotragus niger niger (sable antelope)	+	+
Oryx leucoryx (Arabian oryx)	+	+
Connochaetes taurinus albojubatus (white-bearded gnu)	+	+

Table 1-2 (cont'd)

	0.035M	.07M oxalate
Tragelaphus spekii spekii (east African sitatunga)	+	+
Boselaphus tragocamellus (nilgai)	+	+
Aepyceros melampus rendilus (Impala)	+	+
Antilope cervicapra (black buck)	+	+
Gazella dama dame (dame gazelle)	+	+
G. granti roosevelti (Roosevelt's gazelle)	+	+
Ovis aries (corriedale & suffolk sheep)	+	+
O. ammon musimon (mouflon)	+	+
O. a. f. aries (four horned sheep)	+	+
Capra aegagrus f. hircus (pigmy, mexican hair, Nubian, Cretian, La Mancha goat)	+	+
C. falconeri heptneri (Turkomen markhor)	+	+

lished later in a catalogue for identification. In almost all instances where Hb crystals were formed, morphological differences between crystals were sufficient to permit ready separation at the generic, and sometimes at the species level.

The Hb of primates, as a group, was generally difficult to crystallize. Their crystal production was better at 0.07M oxalate. The pigmy chimp failed to form crystals with the 0.035M solution. A few species would not form crystals with either solution (Lemur, Mangebey, Mandrill, Gelada baboon and raccoon). The clouded leopard and pig formed crystals only with the 0.035M oxalate solution. In general, Hb of Canidae and Perissodactyla formed crystals the most readily followed by Rodentia, Felidae and Artiodactyla and Primates in order of increasing difficulty. Thus, of 100 mammalian species examined here, 95% were successfully crystallized by either reagent and 93% by each of the two oxalate concentrations, at 0.07M (pH 6.8) and 0.035M (pH 6.8) ammonium oxalate.

F. The effectiveness of the reagent on blood meal crystallization

The crystals were identified from blood meals derived 6 hours after ingestion from several mosquito species fed on different hosts (Table 1-3). Crystals obtained from midgut preparations were similar in morphology though sometimes a little slower in appearing (mouse, sheep and cottontail) than crystals obtained from erythrocyte lysates, but were still readily identifiable to source. Mouse erythrocyte lysates crystallized as small needles and hexagonal plates. The needles always appeared first and were sometimes the only crystal form obtained from blood meals. The maximum length of digestion time after which positive identification of the blood meal was possible, was 30 hours with goat and 48 hours with guinea pig, dog and horse.

The buffered (pH 6.8) reagent 0.035M oxalate worked well for 9 of the 11

Table 1-3. A summary of hemoglobin crystals identified from mosquito blood meals after 6 hours digestion.

Host	% Crystallization Success	
	0.21M ammonium oxalate (Washino & Else 1972)	0.035M pH 6.8 ammonium oxalate
Canis familiaris (dog)	100 (16)	100 (41)
Porcellus cavia (cavy)	100 (7)	100 (55)
Mus musculus (mouse)	76 (17)	91 (45)
Capra hirca (goat)	100 (24)	100 (39)
Sus scrofa (swine)	100 (4)	100 (20)
Equus caballus (horse)	92 (27)	100 (20)
Bos taurus (cow)	27 (11)	21 (28)
Ovis aries (sheep)	33 (3)	93 (44)
Homo sapiens (man)	0 (18)	43 (21)
Sylvilagus auduboni (cottontail)	65 (20)	77 (36)
Lepus californicus (jackrabbit)	94 (19)	94 (32)

( ) = replicate numbers

10 species of mosquitoes used include (Anopheles freeborni, Aedes increpitus, A. cataphylla, A. melanimon, A. aegypti, Culex pipiens, C. tarsalis, C. peus, Culiseta inornata and C. incidens.

species tested and improved the crystallization of sheep, mouse, cottontail and man, but not bovine blood meals.

DISCUSSION

Crystallization was more easily attained with the relatively insoluble Hb than with the more soluble ones. Therefore, techniques aimed at reducing the solubility of more soluble Hb's, buffering at human Hb's isoelectric point, the presence of ammonium oxalate and the maintenance of the slides at $4^{o}C$, were used to improve crystallization success. Insoluble particulate matter (e.g., red cell ghosts) appeared to hinder crystallization and was removed by centrifugation.

Although the salt concentration requirement for crystallization varied with the Hb species involved, ammonium oxalate still appeared to be the most effective crystallization agent. It promoted crystallization at low molarity without precipitating other plasma proteins.

Characterization of Hb crystals from various species of mammals and birds has been published as a monograph (Reichert & Brown, 1909). Crystal morphology can be correlated with phylogenetic groups, and Hb's from many different species may be crystallized using essentially similar procedures for each.

The ready separation of certain closely related animals (e.g., bovids and rodents) represents a major accomplishment, since separation of these groups is either difficult or cumbersome by conventional serological means.

Although the specificity of crystal structure is remarkably constant, some heterogeneity of crystal types obtained from a given species was often found. This formation was, however, usually quite minor and never obscured the results. Using identical techniques of preparation from erythrocyte lysates

and from blood meals, the soluble Hb of man formed either small rectangles or needles, that of mouse formed hexagonal plates and/or needles.

O'Gower, (1955) suggested that digestion processes among mosquito species may differ and could, therefore, conceivably influence crystallization. Crystals formed from a given type of Hb, however, were the same regardless from which species of mosquito they came. Thus, the technique should be applicable for most mosquito species.

The possibility of defining a reagent which would permit crystallization of all, or even the vast majority of vertebrate Hb seemed initially to be unlikely. The experience gained from working with diverse Hb derived from animals, however, indicates that 0.035M ammonium oxalate in 0.025 sodium phosphate buffer at pH 6.8 is presently the optimum reagent for use with most mammalian Hb.

Addendum to Experiment 1

To overcome the difficulties encountered with cow blood, several modifications were made in the standard procedure for Hb crystallization. The following briefly summarizes these changes:

1. As an extension on crystallization success of mosquito blood meals with different formulations of ammonia oxalate (Table 1-2), 0.035M and 0.07M ammonia oxalate with high phosphate buffer molarities of 2.0, 1.8 and 1.5 at pH 6.15 $\pm$ .02 and 6.5 were tested specifically to improve test performance against cow meals after a 6 hour digestion interval. Optimum cow crystal results were obtained with 0.07M ammonia oxalate, pH 6.15 $\pm$ .02, 1.5M phosphate buffer. In addition, this high molarity reagent induced greater crystallization success of human blood meals than did the low molarity reagent, but the results were still inconsistent at best. For the high molarity reagent, 1N NaOH and 1M HCl were used to adjust pH with no adverse effects. The addition of ethyl alcohol to the various reagents did not materially improve the crystallization process.
2. Hb from all of the vertebrate hosts listed in Table 1-2 were tested with the high molarity reagent to determine whether or not crystals of different size and/or shape might be produced by the modified reagent and cause confusion. The results were, however, negative.
3. Prior to testing any midgut samples, the standard procedure described previously was modified to take advantage of the results of the high molarity reagent with cow blood meals. Each blood meal was sectioned longitudinally into equal halves with a razor blade. The two halves were placed in separate microtiter well and 10 µl of .025 M buffer

reagent (see 1) was added to one half sample and 10 µl of 1.5 M buffer reagent (see 1) added to the second half sample. Each half midgut was macerated with a small glass pestle and each resulting suspension transferred to a glass slide, allowed to dry at the periphery and a coverslip applied. The margin of the coverslip was sealed within the hour and examined for crystals. Note that the changes also included the elimination of centrifuging the blood meal samples.

EXPERIMENT 2: VARIABILITY OF THE CRYSTAL GROWTH WITH RESPECT TO DIFFERENT MOSQUITOES AND VERTEBRATE HOSTS.

Objective: To study the possible variability of the crystal growth when the Hb crystallization technique is employed with a wide variety of mosquitoes (14 species) with divergent blood meal sources (12 mammalian hosts).

Materials and Methods

Reference crystals were prepared from heparinised whole blood of 12 mammalian hosts as described in Experiment 1. The hosts were man (Homo sapiens), laboratory mouse (Mus musculus), guinea pig (Cavia porcellus), jackrabbit (Lepus californicus), cottontail (Silvilagus auduboni), dog (Canis familiaris), cat (Felis domesticus), horse (Equus caballus), pig (Sus scrofa), sheep (Ovia aries, suffolk or suffolk cross), goat (Capra hircus) and cow (Bos taurus).

Four genera of mosquitoes were used in the experiment. A minimum of ten blood meals for each mosquito species used, were obtained. These were laboratory cultured Aedes aegypti, Ae. albopictus, Culex tarsalis and Cx. pipiens together with field collections of Aedes melanimon, and Ae. nigromaculis, Culex tarsalis, and Cx. pipiens, Anopheles freeborni, Culiseta incidens and Cs. inornata. Mouse and wild rodents were also fed on by Ae. vexans, Ae. cataphylla and Ae. dorsalis.

The mosquitoes fed either directly on the restrained host or through a "natura lamb" membrane (Youngs Rubber Corp.) on freshly extracted heparinized host blood, which was warmed during feeding. The mosquitoes were killed 6 or 24 hours after blood ingestion and their midguts removed. The blood meals were either processed directly or stored in gelatin capsules at -20^{o}C. Mouse blood meals were sampled more frequently, at 6, 8, 12, 16 and 24 hours after ingestion. A minimum of 10 meals was used for each treatment. Microcrystals were

prepared from these blood meals by the method described in Experiment 1. In summary, 10 µl of a 0.035M ammonium oxalate solution in a 0.025M phosphate buffer (pH 6.8) was added to the homogenized blood meal. The mixture was taken up in a 10 µl capillary, one end sealed with clay and centrifuged at 12000 g for 3 minutes. The precipitate and clay were removed, and the drop of clear solution placed on a clean glass slide and left to dry until a definite dried ring formed around the edge of the drop. A coverslip was placed over the drop and the slide examined for developing crystals. The coverglass was sealed with permount within three hours of preparation and the slides stored at 4°C.

Results

The relative success of crystallization of blood meal Hb from the 12 host species is given for Aedes species, Culex spp., An. freeborni and Culiseta spp. (Table 2-1). Crystallization success rate varied from 0% to 100% depending upon the host involved and was greater at 6 hours than at 24 hours after ingestion. The most readily crystallizable Hb were the most insoluble ones of guinea pig, dog, horse, and goat. For example, guinea pig crystals could still be reliably identified 36 hours after blood ingestion by A. aegypti, even though by this time, the crystals were quite small (Table 2-2). The success of Hb crystallization of the blood meals from these four hosts was not affected by the different mosquito species.

Crystals were not quite as reliably produced from pig, jackrabbit and sheep. The success rate with these animal species was 86-95% at 6 hours after ingestion and 78-90% at 24 hours. The reproducibility of crystallization was lower with pig and sheep Hb from Ae. nigromaculis meals at 6 and 24 hours after ingestion and for pig from Ae. vexans 6 hour meals. This mosquito species had

Table 2-1. Relative success of the Hb crystallization technique for identification of known hosts from blood meals of *Aedes*, *Culex*, *Culiseta* and *Anopheles* mosquitoes, at 6 and 24 hours after ingestion.

HOST	*Aedes* species[2]		*Aedes nigromaculis*		*Culex* species[3]		*Anopheles freeborni*		*Culiseta* species[4]	
	6	24	6	24	6	24	6	24	6	24
Homo sapiens	28	15	0		0(4)	0	0	0	0(5)	
Mus musculus	92	0	27	0	92	0	100	20	55	0
Cavia porcellus	100	100	95	90	100	100	100	100	100(7)	100(9)
Lepus californicus	100	83	93		90		100	90		
Sylvilagus auduboni	75	54	36	18	100	66	13	10	44	46
Canis familiaris	100	100	100	100	100	100	100	100	100	100
Felis domesticus	50	77	60	9	27		60	58	73	30(8)
Equus caballus	100	100	100	91	96	100	100	100		100
Sus scrofa	100	100	8	0	20	87	100	100	100	50
Ovis aries	100	84	13	9		100(9)	100	88	70	54
Capra hircus	100	100	100	100	100	100	100	100	100	91
Bos taurus	16	0			0		0	0	0	

[1] minimum of 10 meals for each species of mosquito except where indicated in parentheses.

[2] *Aedes aegypti*, *Ae.* melanimon and/or *Ae. albopictus*.

[3] *Culex tarsalis* and/or *Cx. pipiens*.

[4] *Culiseta inornata* and/or *Cs. incidens*.

Table 2-2. Guinea pig Hb crystal production 6 to 48 hours after ingestion.

Hours after ingestion	% success	
	Ae. aegypti	Cx. pipiens
6	100 (15)	100 (21)
24		100 (11)
36	100 (11)	45 (11)
48	50 (10)	

( ) replicate no.

no apparent effect on the crystallization of jackrabbit blood meals at 6 hours. In Culiseta inornata crystal production was reduced for sheep blood meals after 24 hours ingestion.

Hb crystals were formed less easily from cottontail blood meals in all mosquito species and the success of crystallization declined as digestion progessed. As with pig, crystals were more difficult to obtain from Ae. nigromaculis and Ae. vexans blood meals, even at 6 hours, than from any other aedine species.

The success of Hb crystallization of mouse blood was measured for different mosquito species at a series of time intervals after ingestion (Table 2-3). Crystallization was unreliable 6 hours after ingestion. Results with Anopheles, Culex and some aedine species with mouse blood were better than those obtained from Culiseta and other aedine spp. Ae. nigromaculis blood meals had a consistently lower crystallization success. The reduction in Hb crystallization success rate with increasing time after blood ingestion was common to all mosquito species studied. After 12 hours, the results were poor for all mosquitoes except with Culiseta (45% after 24 hours), but Cx. tarsalis and An. freeborni showed a slower reduction in success with time. Certainly in the case with Culiseta it could reflect meal size. The data is similar, but less complete for cat. Although the present reagent produced its crystals reliably from fresh cow and human whole blood samples, crystal production from these same hosts, as blood meals, was poor and inconsistent.

We also compared the success of crystallization of blood meals from Ae. aegypti and field collected Aedes species on a marsupial and a number of species of California rodents (Table 2-4). Didelphis and Neotoma species crystallized readily; Peromyscus, Dipodomys and Eutamias were crystallized somewhat less readily. Crystals from a species within a genus, (e.g., Dipodomys,

Table 2-3. The success of hemoglobin crystallization of mouse blood at various time intervals after ingestion.

	Percent success of crystallization with hours after ingestion.*				
Mosquito sps.	6	8	12	16	24
Aedes aegypti		64(13)	0(18)	0(12)	0(12)
Ae. melanimon	92(12)		60(10)		0(12)
Ae. nigromaculis	27(15)	25(20)	0(13)	10(10)	9(19)
Ae. increpitus	75(4)		0(4)		
Ae. cataphylla	30(10)				7(13)
Ae. vexans	60(10)				0(11)
Culex tarsalis	92(12)	66(12)	30(10)	27(11)	12(8)
Cx. peus	77(9)				0(15)
Cx. pipiens	50(4)	16(6)	0(1)		0(2)
Anopheles freeborni	100(10)				20(10)
Culiseta inornata	50(10)	80(5)	0(2)	0(8)	0(21)
Cs. incidens	60(10)	0(3)		0(1)	43(7)
Totals	64(106)	44(59)	16(58)	10(42)	5(130)

* ( ) No. ♀♀ tested.

Table 2-4. Hemoglobin crystallization success for identification of wild rodents and opossum from mosquito blood meals, 6 hours after ingestion.

Host species	*Aedes aegypti*	other aedine species	*Culex tarsalis*
Dipdelphis marsupialis (opossum)	100(25)		
Eutamias sp. (chipmunk)	13(9)		
Rattus rattus (roof rat)		80(5)[4]	
Neotoma cinerea (bushy tail woodrat)	100(12)	100(5)[1]	
N. lepida (desert woodrat)	90(10)	100(5)[3]	
N. fuscipes (dusky footed woodrat)	100(10)		89(9)
Peromyscus truei (Pinon mouse)	91(11)	84(12)[1] 60(5)[2]	
P. maniculatus (Deer mouse)	83(6)	75(20)[2] 0(9)[4]	84(12)
P. crinitis (canyon mouse)	14(7)		
Dipodomys ordi (ord kangaroo rat)	63(8)		
D. heermanni (Heermann kangaroo rat)	100(4)	0(5)[4]	
D. panamintinas (Panamint kangaroo rat)	200(24)		
D. merriami (Merriam kangaroo rat)	60(5)		
D. microps	40(5)		

replicate numbers ( )

[1] *Ae. dorsalis*
[2] *Ae. cataphylla*
[3] *Ae. vexans*
[4] *Ae. nigromaculis*

Neotoma and Peromyscus) were similar in form, yet crystallization success within the genus varied greatly, particularly with Dipodomys and Peromyscus genera. This indicated differences in Hb solubilities which vary within a genus, but also between species.

Discussion

Our laboratory evaluation showed that Hb from different vertebrate species exhibit a range of solubilities. Hb solubility and to a lesser extent, the mosquito species involved, appear to govern the success of the crystallization technique for individual vertebrate host species. Therefore, the crystallization success of any blood meal containing soluble Hb may be considerably less in some mosquitoes, e.g., Ae. nigromaculis. Conversely, with blood meals containing insoluble Hb, little difficulty is encountered regardless of the mosquito species involved.

Development of a second reagent more specific to the soluble Hb (e.g., cow and man) is necessary for the eventual success of this technique. In the last stages of Experiment 1, a reagent specifically for bovine-originated blood meals was developed and evaluated. It was concluded then to divide one blood meal and use two reagents per blood meal to accomodate Hb of different solubilities which would enhance the usefulness of this technique.

EXPERIMENT 3: ELECTROPHORESIS AND ISOLLECTRO-FOCUSING

Objectives:

1. Electrophorectic studies were conducted to observe more precisely the pattern of mosquito blood meal digestion by utilising different host species and different mosquito species. This investigation was to determine changes in Hb concentration and state in order to elucidate difficulties experienced with blood meal crystallization with certain vertebrates (e.g., cattle) and with certain hematophogous insects (e.g., A. nigromaculis).
2. The relationship between electrophoretic values of Hb and polymorphic Hb with their crystal morphology was studied.
3. The effect of storage of frozen vertebrate blood on its electrophoretic and crystallization properties was examined.

Material and Methods

General

An Aardvark instruments unit was used for the polyacrylamide gel electrophoresis studies. This slab gel system allowed up to 24 samples to be run simultaneously on the same gel. A tube type electrophoresis system was used for isoelectrif focusing, as this reduced the quantity of expensive ampholytes required. Electrophoretic techniques were developed to suit our specific requirements (see methodology section). Blooded mosquitoes were obtained by either feeding the mosquito on the host or host blood via a membrane or by enema.

Electrophoresis

Slab gel

An Aardvark electrophoresis apparatus was used in this study. This was

a slab gel system which allowed up to 24 samples to be run simulatneously on the same gel, allowing accurate intragel comparisons. A Buchler power supply (model 3-115) provided the necessary potential. A discontinuous system of 2.5% acrylamide stacking gel and 7.5% acrylamide running gel was adopted to improve the clarity of the bands.

All electrophoresis purity grade chemicals were used except for acrylamide (Sigma Chemcial Company no. A-8887) which was recrystallized in chloroform to increase purity. All water used for gel and buffer was double deionised. Water at room temperature was run through the apparatus during gel polymerization and electrophoresis. The gel comb was removed after the gel had hardened and tris-glycine buffer (pH8.3) was poured into both electrode chambers.

The samples were prepared by mixing Hb, 10% sucrose=bromophenol blue solution and a hemolysate reagent (KCN in EDTA, Helena Laboratory) in the ratio 1:1:1. Bromophenol blue served as the solvent front marker. The samples were centrifuged and 8 µL applied to the gel under the buffer solution. A known characterized blood sample (A_I Helena laboratory) was run as a standard on each gel to allow intergel comparisons. The gel was run with constant current at 45mA for four hours. The migration distance of the various Hb bands and the solvent front were measured. The ratio of these distances gave the Rf. These were standardized against HbA_I Rf (Rf_A) for that gel.

Isoelectrofocusing

Tube gel electrophoresis was used as it required smaller quantitites of the expensive ampholyte. The apparatus used 13, 5mm (I.D.) x 100mm tubes. A 40% (w/v) ampholyte solution (pH6.8) (LKB Produkter, Sweden) was used to establish the pH gradient across the gel tubes. The other reagents were the

same as those used for the slab gel.

The procedure of Wrigley (1971) was followed. Polymerization was induced by exposing the gel solution, which contained Riboflavin to a UV light source for one hour. The anode chamber was filled with a basic solution and the cathode by an acidic one. The samples consisted of 10µL HbA_{1A} the internal standard; 10µL Hb sample; 10µL hemolysate (pH 9.3) and 10µL 10% sucrose solution. 20µL sample was applied to the top of the polymerised gel. One gel was left blank to establish the pH gradient. After the run, this blank gel was cut into 5mm section, each slice was soaked overnight in 1ml of water and the pH gradient determined. A control without the Hb sample was also included. The gels were run for 4 hours at 0.1 watt/tube with constant voltage (to discourage heating). Band sharpness was enhanced by increasing the voltage to 0.04 watts/tube during the last 15 minutes.

Reagent preparation

Slab gel

Separating gel (7.5% acrylamide)

a. 48 ml 1N Hcl

   36.3 gm Tris (hydroxymethyl) amino-methane (Tris)

   0.23 ml N,N,N,N - tetramethylene diamine (TEMED) (Bio-Rad) were mixed together and diluted to 100ml (pH8.8-9.0).

b. 30 gm acrylamide

   0.735 gm NN - methylene bisacrylamide (Bis) (Eastman Kodak Co.) were mixed together and taken up to 100 ml in H_2O.

c. 0.11 gm of ammonium persulphate were dissolved in 75 ml of water, just prior to polymerization.

   37.5 ml of solutions a,b and 75 ml of solution c were mixed with water

to make a total of 150 ml. The solution was immediately filtered by suction and degassed. It was then pured into the gel space. 4 mm of water was layered on top of the gel. which was carefully removed after polymerization.

Stacking gel(2.5% gel)

a. 5.98 gm Tris

0.46 gm TEMED were mixed with approximately 48 ml IN HCl (pH6.7) and diluted to 100 ml with H_2O.

b. 20 gm acrylamide

2.5 gm Bis were mixed and taken up to 100ml in H_2O.

c. 0.07 gm ammonium sulphate was mixed with 12.5 ml water just prior to use.

d. 40% sucrose solution.

These solutions were mixed in the ratio a:b:c:d, 1:1:2:4. The solution was filtered as before and pured into the remaining gel space. Polymerization takes place in less than 20 minutes.

Buffer solutions

18 gm Trizma base

86.4 gm glycine were mixed and made up to 6 liters with water and pH adjusted to 8.3.

Iso-Electrofocusing gel preparation

a. 30 gm acrylamide

1 gm bis in 100ml H_2O and filtered.

b. 0.5ml ampholyte solution (pH6.8 LKB Produkter Sweden) 1.17ml 0.015% (W/V riboflavin were mixed with water to make 15ml of solution. A ratio of 1:3 (a:b) constitutes the working solution which was stirred

under vacuum for 10 minutes. The solution was shaded from light at all times prior to polymerization.

Base lock solution

500ml 0.4% (v/v) ethylene diamine

Acid lock solution

500ml 0.2% sulphuric acid

Results and discussion

1. As expected, a decrease in Hb quantity was observed with increased digestion time for both A. aegypti (Fig. 3-1) and A. nigromaculis. It was difficult to compare the two; A. nigromaculis actively eliminated many blood droplets from its alimentary tract. Attempts to reduce this elimination with an anal seal resulted in high mosquito (A. nigromaculis) mortality within five hours.

   The more sensitive detector system of isoelectric focusing resolved differences in composition of the Hb at various times after enema insertion for both A. aegypti and A. nigromaculis cow blood meals and A. nigromaculis mouse blood meals. In each of these examples, the number of Hb bands increased in the blood meals compared with fresh blood. It was noted for bovine (jersey) blood, that bands B and C (modified Hb) increased with time in the mosquito at the expense of band A (native Hb) (Fig.3-2). The new bands formed were more acidic than the original native protein. Ingested blood bands were more diffuse than fresh blood. This was evident even half an hour after ingestion. Although further replication of these experiments are desirable, it appeared that cow blood was rapidly denatured. After 10 hours digestion in both mosquito species, less than half of the

Hb was in its original form. This decrease in native Hb concentration may lessen the success potential for crystallization especially with soluble Hb. The rate of Hb denaturation appeared similar for both mosquito species. Since the abdominal pore was sealed to prevent blood elimination in A. aegypti, comparison with A. nigromaculis was difficult. It was interesting to note that on an earlier citrate agar gel (Helena laboratory test) that two Hb bands were noted after 24 hours ingestion of bovine (Angus) Hb in A. melanimon instead of one Hb band for the whole blood sample.

Further studies are essential to compare the insoluble with soluble Hb and to delineate further the effect of mosquito, host blood or both on Hb digestion and crystallization. It appeared however, from the results of our preliminary isoelectric focusing experiment that Hb was altered quite rapidly and it is this rapid loss in concentration of the soluble cow hemoglobin which plays a part in the reduction of crystallization success with the present reagent.

2. Table 3-1 gave the electrophoretic values (Rf/Rf^{*}_{A}) as determined on the slab gel. Further replication of each species was desirable, but our blood samples were limited to one or two samples per species. Hb polymorphism appeared in individuals from all mammalian orders, and was commonly observed among the primates, artiodactyls and birds. It is generally accepted that genetic distance can be measured by similarities between Hb electrophoretic mobilities or crystallization patterns would be expected between close phylogenetic groups. Conversely, differences would be expected between widely separate groups, and our results tended to confirm this. The canidae have similar

*Rf_A = distance moved by standard Human Hb_{AI}

Fig. 3-1

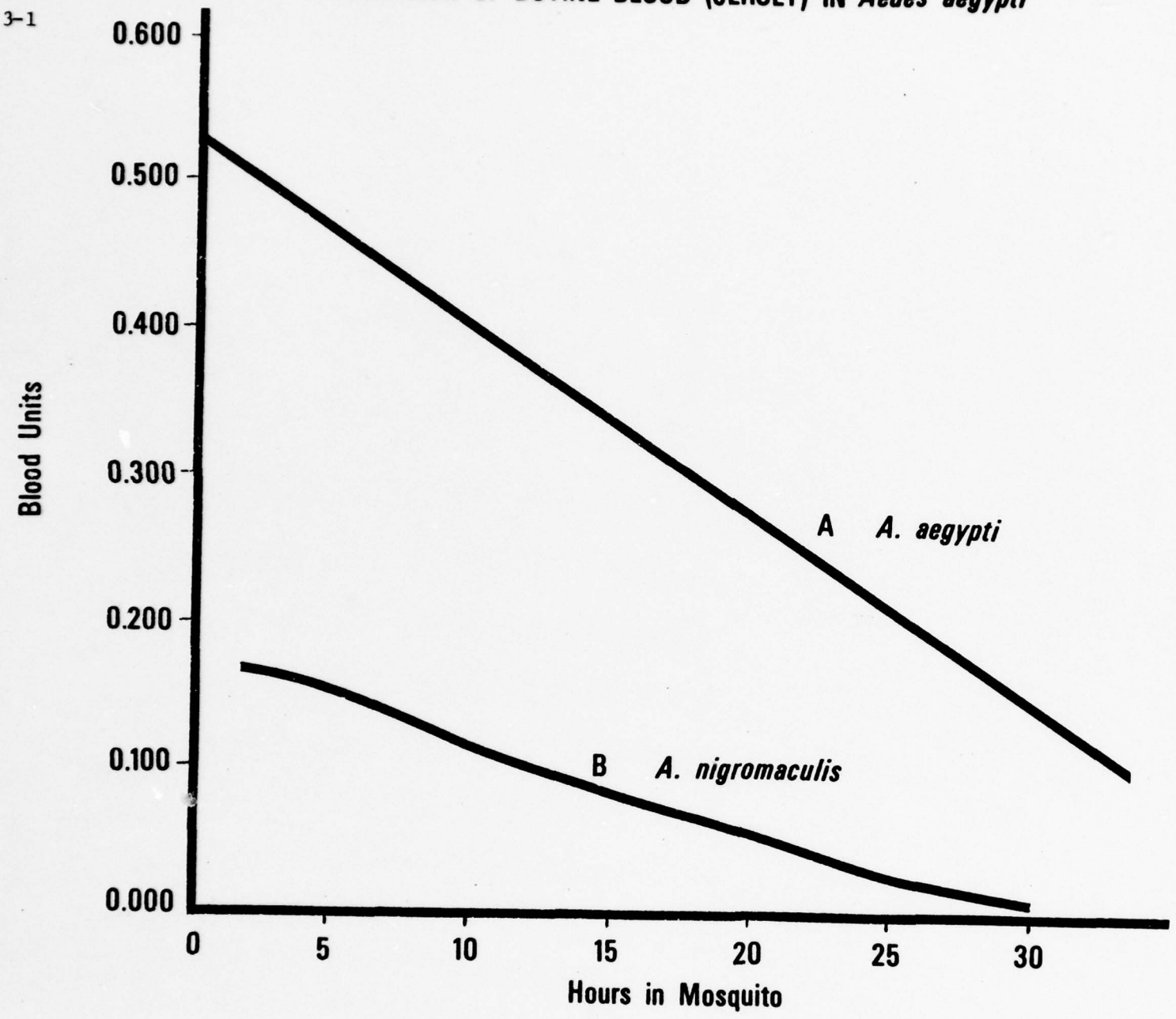

DIGESTION OF BOVINE BLOOD (JERSEY) IN *Aedes aegypti*
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Blood Units
A *A. aegypti*
B *A. nigromaculis*
0
5
10
15
20
25
30
Hours in Mosquito

Fig. 3-2a

CHANGES IN HB OF COW BLOOD AFTER INGESTION BY *Aedes aegypti* OBSERVED WITH ISOELECTRIC FOCUSING. (MEAN OF 2 REPLICATES)

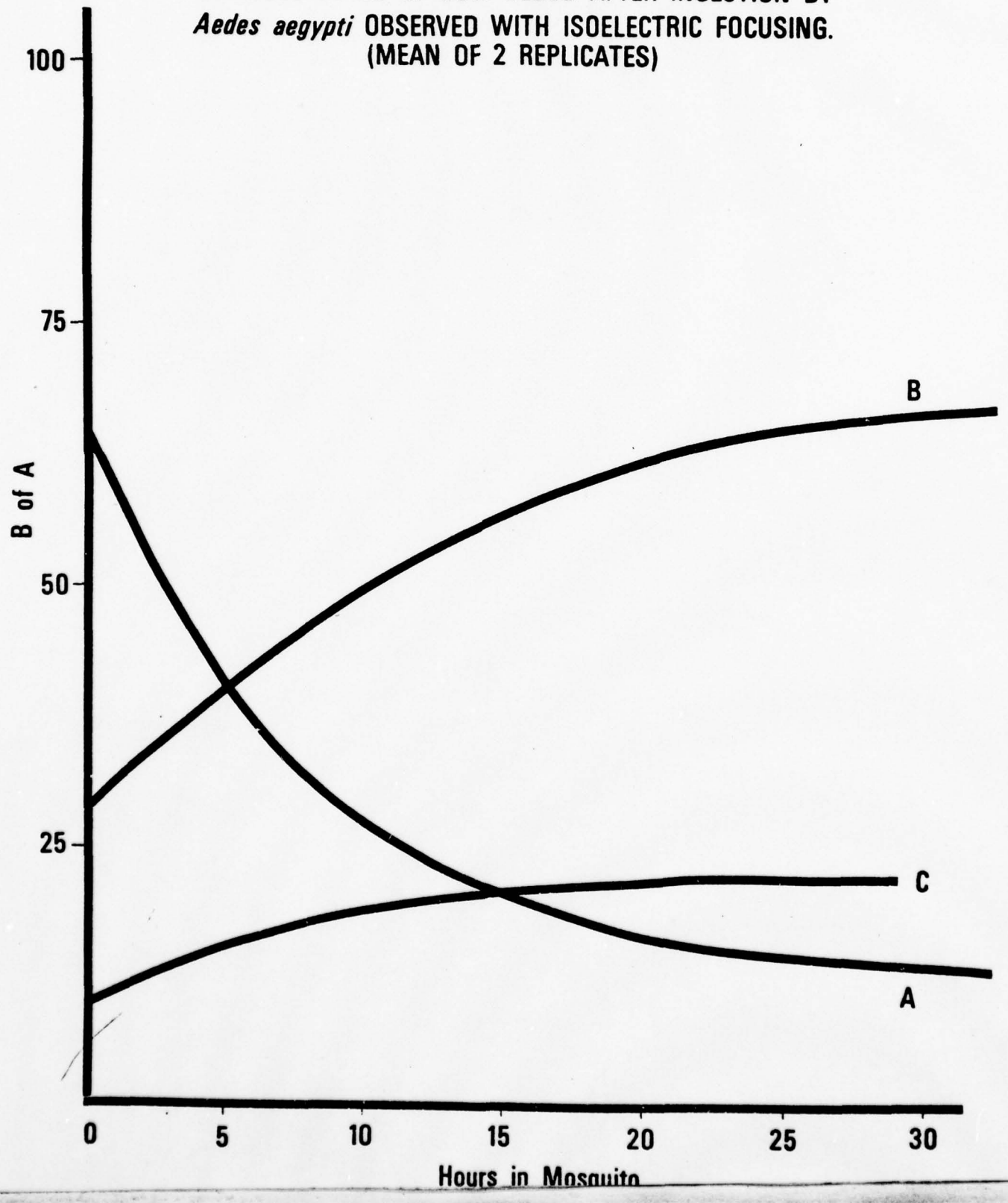
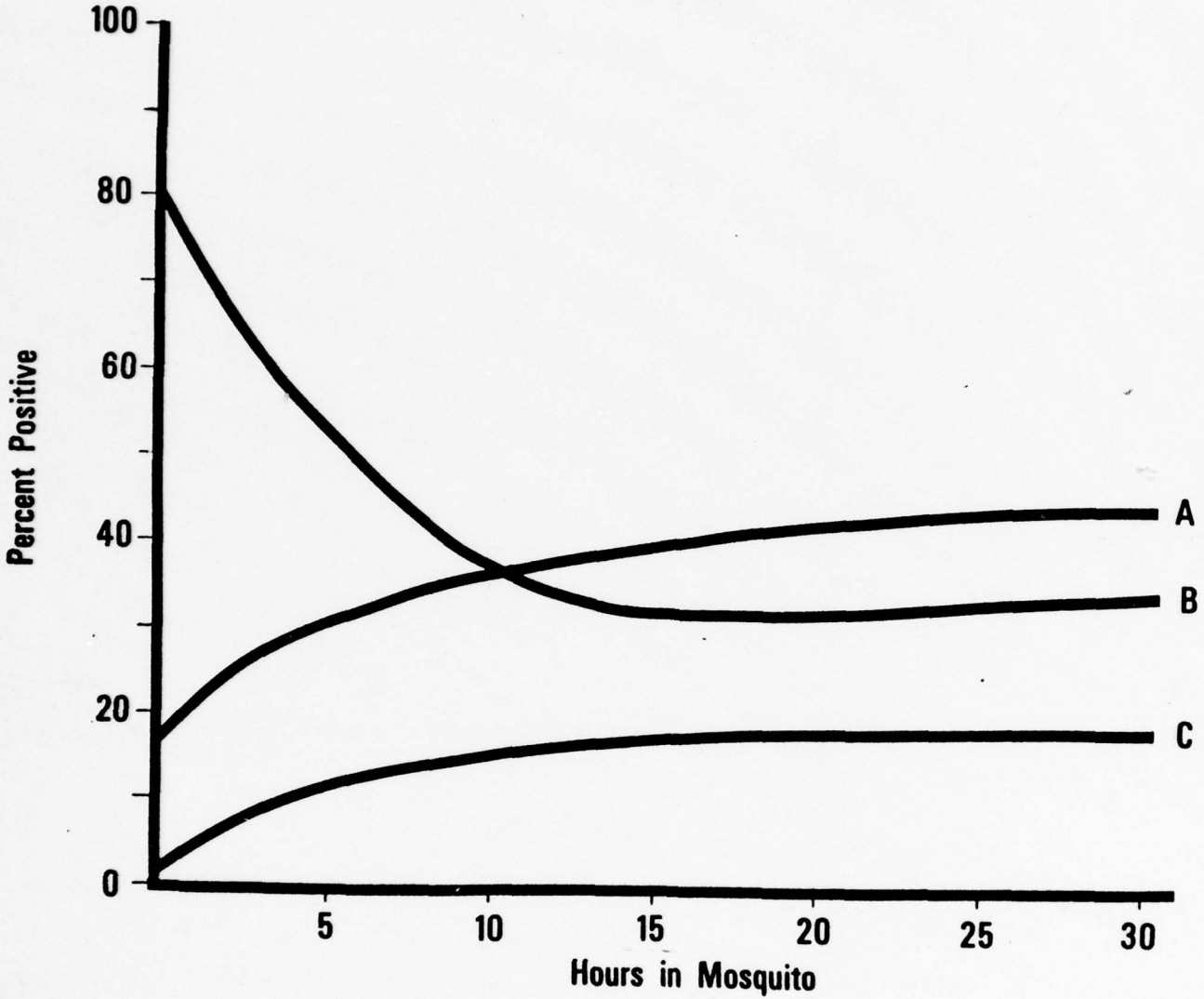


Fig. 3-2b

CHANGES IN HB OF COW BLOOD AFTER INGESTION BY *Aedes nigromaculis* OBSERVED WITH ISOELECTRIC FOCUSING



Rf/Rf_A at 0.82-0.87 and typically insoluble Hb. Crystal structure was found to be similar with long prismatic needles. Similarly, the four Camelidae representatives studied here have two Hb which also have similar Rf/Rf_A (0.57-0.65 & 0.73-0.78). However, dromedary Hb was very soluble and difficult to crystallize compared with lama Hb. Crystal morphology and Rf/Rf_A were similar within other genera, e.g., Primates; Cacajao, Cercopithecus and Macaca, Rodentia, Dipodomys and Peromyscus, and Equidae. Birds often have two Hb types, one of which was usually much slower moving than any mammalian Hb.

On the basis of this preliminary work, there appears to be no correlation between electrophoretic mobility, solubility or crystal morphology. For example, opossum (Rf/Rf_A 1.1) rhinoceros (Rf/Rf_A 1.2) and tapir (Rf/Rf_A 1.3) had similar superficial crystal morphology, but their Hb solubility and Rf/Rf_A were different between the main taxanomic groups. As mentioned previously, the Indian-Chinese sika hyrax also have a similar crystal morphology; their Rf and solubilities however, were very different.

No correlation was found between crystal morphology and electrophoretic mobility between the main taxanomic groups. Many different species (e.g., Human A, white ukari, macaccas, gelada baboon, southern dusky langur, goat, cow, bison, and domestic cat) have the same mobility, but different crystallization properties. Thus, electrophoresis alone has limited value as a diagnostic tool for species identification.

3. No changes in the electrophoretic properties of cow blood were observed even after prolonged storage (1 year). Crystallization, however, was less reliable than with fresh samples.

Table 3-1. Accumulative list of Animal Species from which Rf/Rf_A have been found.

MARSUPIALS	Rf/Rf_A	PI
Didelphoidea		
Didelphis marsupialis	0.98	7.08
Macropodiadae		
Dendrolagus matsechiei	0.95	7.04
D. goodfellowi goodfellowi	0.73,0.89	
Protemnodon agilis jardini	1.05	
Walleroo	1.01	
Thylogale parma	1.01	
Dasyuroidea		
Sarcophilus harrisi	0.62, 0.74	
EDENTATA		
Dasypodidae		
Dasypus novemcinctus	1.01	
PRIMATES		
Lemuridae		
Lemur variegatus	0.98, 1.13	
Varecia variegatus ruber	0.78*, 1.04*, 1.16	
V. variegatus	0.84, 1.05	
Cebidae		
Ateles sp.	0.80, 1.07	
Cacajao calvus calvus	1.00	
C. calvus rubicundus	1.05	
Cercopithecidae		
Macaca arctoides	0.83, 0.95, 1.00	
M. rhesus	1.00	
M. cynomologous	1.00	
M. sylvanus	0.95, 1.00	
M. nemistrina	0.89*, 1.19	
M. silenus	1.31	

	Rf/Rf_A	PI
Papio anibis	1.06	
Comopithecus hamadryas	0.66, 0.99	
Cynopithecus niger	0.67, 0.96	
Erythrocebus patas patas	0.98	
Cercopithecus nictitans schmidtii	0.75, 0.99	
C. aethiops	0.70, 1.00	
Theropithecus gelada	1.00	
Presbytis obscurus obscurus	1.00, 1.06	
P. entellus	0.99	
Pygathrix nemaeus nemaeus	0.98	
Nasalis larvatus orientalis	0.98	
Hylobates concolor gabriellae	0.91	
Pongidae		
Pan paniscus	1.10	
P. troglodytes verus	1.08	
Pongo pygmaeus pygamaeus	0.83, 0.95	
Hominidae		
Homo sapiens type A	1.00	
sickle cell (old blood)	0.53, 0.78	
RODENTIA		
Sciuromorpha		
Sciuridae		
Sciurus griseus	0.80, 1.03	
N. E. squirrel	0.80, 1.03	
Eutamias sp. A_3	0.90	
Spermophilus beldingi	0.85, 0.95	
Myomorpha		
Heteromyidae		
Dipodomys ordi	1.12	
D. microps	1.09	
D. panamintinas	1.07	
D. heermanni	1.07	

	Rf/Rf_A	PI
Cricetidae		
Peromyscus truei	0.81, 1.05	
P. crinitis	0.84, 0.93	
P. maniculatus	0.84, 0.97	
Muridae		
R. norvegicus	1.08	
Hystricomorpha		
Erethizoa dorsatum	1.31	
Caviidae		
Cavia porcellus	0.82, 1.15	
Lagomorpha		
Lepus sp.	0.90	
CARNIVORA		
Canidae		
Canis latrans	0.86	
C. familiaris	0.82	7.06
C. lupes	0.86	
C. aureus syriacus	0.85	
Chrysocyon brachyurus	0.87	
Speothas venaticus venaticus	0.87	
Procyonidae		
Ursidae		
Ursus sp.	0.88	
Mustelidae		
Mephitus mephitus	0.88	
Felidae		
Felis domesticus	1.00	
F. concolor	1.05	
F. silvestris caudata	1.11	
F. serval ingridi	1.22	
Panthera leo	1.25	
P. tigris tigris	1.19	
P. tigris altaica	1.19, 0.91*, 0.76*	
P. pardus japonensis	1.10	

	Rf/Rf_A	PI
P. pardus delacouri	1.19	
Acinonyx jubatus	1.03	
Genetta genetta felina	1.03	
PAENUNUGULATA		
Hyracoidae		
Procavia capensis capensis	1.33	
PERISSODACTYLA		
Equidae		
Equus caballus	1.12, 1.29	
E. przewalski przewalski	1.12, 1.29	
Asinus africanus f. asinus	1.11	
Hippotigris zebra hartmannae	1.14	
Rhinocerotidea		
Ceratotherium simum simum	1.23	
Rhinoceros unicornis	1.22, 1.47	
Tapiroidea		
T. bairdii	1.29	
ARTIODACTYLA		
Suiformes		
Suidae		
Sus scrofa	1.00	
Tayassuidae		
Tayassu tajacu sonoriensis	1.00, 1.11	
Tylopoda		
Camelidae		
Camelus ferus f. dromedarius	0.63, 0.76	
Lama perusna	0.63, 0.76	
L. guanicoe guanicoe	0.65, 0.78	
L. guanicoe f. glama	0.57, 0.73	
Ruminantia		
Cervidae		
Odocoileus hemionus	1.21	
Rangifer tarandus	1.12	
Muntiacus reevesi reevesi	0.95, 1.11	

		Rf/Rf_A	PI
Cervus elaphus sibiricus		1.14	
C. nipon pseudaxis		1.05, 1.22	
C. canadensis		1.14, 1.52	
Antilocapriadae			
Antilocapra americana americana		0.84	
Bovidae			
Bos taurus	Jersey	0.97	
	Hereford	0.98	
	Gurnsey	1.00	
	Angus	1.00	
	Holstein	1.00	
	Cow		7.1
Beefalo (13/16 cow, 3/16 bison)		1.07	
Bison bison bison		0.90, 1.00	
B. b. bonasus (newborn)		1.11, 1.22	
Hippotragus niger niger		0.71*, 0.97	
H. equinus cottoni		1.14	
Oryx leucoryx		1.07	
Connochaetes taurinus albojubatus		0.61*, 0.89	
C. gnou		0.63*, 0.89	
Tragelaphus spekii spekii		1.32	
Taurotragus oryx pattersonianus X *Tragelaphus s. strepsiceros*		1.08	
Boselaphus tragocamellus		0.71*, 0.96	
Aepyceros melampus rendilus		0.81, 0.89	
Antidorcas marsupialis		0.89	
Antilope cervicapra		1.17	
Damaliscus dorcas dorcas		0.87	
Gazella dama dama		0.82, 0.84	
G. granti roosevelti		0.73, 0.92	
Cephalophus silvicultor silvicultor		0.81*, 1.11	
Ovis aries corriedale (white face) suffolk (black face)		0.95, 1.13 0.98	
O. a. f. aries		1.00	
Capra aegagrus f. hircus (pigmy, mexican hair, La mancha goat, Nubian)		1.00	

	Rf/Rf_A	PI
C. ibex severtzovi (newborn)	1.08	
Hemitragus jamlahicus	0.81, 0.89	
AVES		
Rheidae		
Pternocnimia pennata pennata	0.35, 0.54*, 0.84*, 0.95	
Falconiformes		
Buteo jamaicensis	0.48	
Aquila chrysaetos	0.45, 0.71	
Falco sparverius	0.29, 0.93	
Circus cyaneus	0.54, 0.76	
Galliformes		
Phasianus colchicus	0.51, 0.68	
Meleagris gallopavo	0.49, 0.71	
chicken		7.9, 7.75
Passeriformes		
Corvus corvax	0.29, 0.69	
Pica nuttalli	9.57	
Agelaius tricolor	0.50	
Columbiformes		
REPTILIA		
Chrysomys scripta elegans	0.19, 0.46, 1.43	
Lampropeltis getulus californiae	0.55	

* faint bands

EXPERIMENT 4: OTHER CONSIDERATIONS IN THE PRACTICAL APPLICATION OF THE CRYSTALLIZATION TECHNIQUE.

1. Preliminary work was undertaken to identify multiple blood meals within a single gonotrophic cycle in mosquitoes. The results are summarized in Table 4-1. The results varied with different combinations of hosts used. Some samples, e.g., Mus/cavy and horse/chicken formed hybrid crystals, as well as one or both of the individual crystal forms. Most blood mixtures were negative. Hb has been observed to dissociate under certain conditions (e.g., different pH) and recombine to form a new hemoglobin species (hybrid). Identification of multiple meals may be possible if the constituent partial meals could be separated from the whole meal before crystallization. Considerable work is necessary to more completely assess the usefulness of the crystallization technique in identifying multiple blood meals.

Table 4-1. Summary of Hb crystallization success from blood meals taken by mosquitoes after having fed on two or more hosts.

Blood meal Combination	No. of replicates	Crystal pattern
Cavy/man	7	Cavy, negative, hybrid
Cavy/mus	17	Mus, cavy, hybrid
Chicken/cow	1	Negative
Horse/chicken	1	Horse, negative hybrid
Horse/cow	6	Horse, cow
Horse/cow/chicken	1	Negative
Horse/sheep	5	Horse, sheep
Microtus/Dipodomys	1	Negative
Microtus/man	2	Negative
Quail/mus	5	Negative
Sheep/cow	5	Negative, cow

Table 4-2. Hb crystallization success from equal mixtures of Hb lysate samples.

Blood meal Combination	No. of replicates	Crystal pattern
Horse/man	4	Horse, negative
Horse/cow	4	Horse, negative

2. Blood meals of hematophagous arthropods other than mosquitoes were studied by the crystallization technique. These arthropods included the ticks Rhipicephalus sanguinous, Dermacentor variablis and Ornithodoras coriaceus, the bed bug Lecimex larius. Dog blood was identified in the reduced form from the tick, R. sanguinous 30 days after feeding. Guinea pig crystals were readily identified from the bed bug 24 hours after blood ingestion from D. variablis 20 days and O. coriaceus 33 days after feeding. Laboratory studies with the tsetse fly (Glossina Morsitans) are still imcomplete. Our preliminary observations indicate that further work should be conducted to follow through on the possible application of the technique for studying vertebrate host feeding patterns of arthropods other than mosquitoes.

EXPERIMENT 5: LABORATORY AND FIELD EVALUATION OF THE HEMOGLOBIN CRYSTALLIZATION TECHNIQUE

Objectives:

1. To "blind" test the Hb technique in the laboratory by having blood meals of known source and digestion time, identified as unknowns by a person not having any prior knowledge of the test material;
2. To further test the efficacy of the Hb technique, identify blood meals of field-collected mosquitoes by both the precipitin and Hb tests.

Blind Laboratory Test

Material and Methods

Laboratory-reared Aedes aegypti were induced to take blood meals from cow, dog, man and rabbit. Blood meals from horse and sheep were via membrane feeding of heated heparinized blood. The fully, blood-engorged mosquitoes were subsequently maintained at room temperature, and lots of females were killed at 6, 12 and 24 hours digestion time. The midgut was removed from each mosquito and stored at -70°C in gelatinous capsules within a glass vial. In both blind tests, the glass vials were coded and presented to the examiner as an unknown. Hemoglobin was crystallized using the split-abdomen proceudre utilizing .025M and 1.5M phosphate buffer in 0.07M ammonia oxalate as described in Experiment 1.

Results and Discussion

The results in the 1st and 2nd blind tests are summarized in Tables 5-1 and 5-2. Blood meals from host animals with the least soluble Hb were identified most readily. Horse blood meals were all correctly identified (24 of 24) irrespective of time; scores in dog blood meal identification was relatively

high (18 of 23), but still disappointing since dog Hb crystals are characteristically easy to produce and quite distinct. The use of the high molar reagent from cow blood produced a perfect score (8 of 8) at the 6 hour interval, but blood meals digested for longer periods could not be identified with either reagents. The poor success in identifying blood meals from man and to a lesser extent, laboratory rabbit, was not totally unexpected due to some degree of inconsistency in previous work.

Neither scores matched the earlier results of blind tests involving 5 (100%) and 31 (97%) unknowns (Washino and Else 1972). The results of the blind tests were generally not encouraging. Unless marked improvement can be made with cow blood meals with digestion time of 12 or more hours and human blood meals at any time intervals, it is difficult to justify continuing this line of investigation. In contrast to the majority of the vertebrate host blood studied, the major difficulty of cow and human blood is not the incorrect identification of the Hb crystals, but the inconsistency of producing the actual crystals. Once the crystallization process takes place in these two hosts Hb, identification is not as difficult as the blind tests might indicate.

Table 5-1. Identification of mosquito blood meals by a person not knowing the blood meal sources (Blind test 1).

hrs. post-feeding	no. blood meals correctly identified/no. tested						
	Cow	Man	Dog	Sheep	Rabbit	Horse	Total
6	4/4	0/2	3/4	1/2	1/1	4/4	13/17
12	2/4	1/2	4/4	0/1	0/1	4/4	11/16
24	1/4	1/2	2/4	0/2	0/1	4/4	8/17
Total	7/12	2/6	9/12	1/5	1/3	12/12	32/50

Table 5-2. Identification of mosuqito blood meals by a person not knowing the blood meal sources (Blind test 2).

hrs. post-feeding	no. blood meals correctly identified/no. tested					
	Cow	Man	Dog	Sheep	Horse	Total
6	4/4	2/5	2/3	0/0	4/4	12/16
12	1/5	0/3	3/4	0/1	4/4	8/17
24	0/4	0/3	3/4	0/2	4/4	8/17
Total	5/13	2/11	9/11	0/3	12/12	28/50

Precipitin vs. Hb crystallization tests

Materials and Methods

From June through 1973, midgut samples of field-collected, blood engorged Anopheles freeborni were routinely divided into two equal lots from any sampling site and stored at -70°C. Approximately half of the 2948 midgut samples collected in 1973 were subsequently precipitin tested (Tempelis and Lofy 1963) within a year by Dr. Tempelis at the University of California, Berkeley. It was originally intended to identify the other half by the Hb technique during the same approximate period (1974), but continuing difficulty with developing a suitable reagent against cow and man delayed the testing until early and mid-1977. Slightly over half (860) of the 1540 midgut samples were completed in time to be included in this report. The split-abdomen procedure with .025M and 1.5M phosphate buffer in .07M ammonia oxalate was used for Hb crystallization.

Results and Discussion

A meaningful comparison of the results is difficult until such time the unfinished samples to be tested by Hb crystallization are completed. If, however, the trend on the feeding pattern remains relatively the same, the crystallization method will compare unfavorably with the precipitin test (Table 5-3). The percentage of mosquitoes reacting to any host with the Hb method (51.6 per cent of 860) is significantly lower than the results of the precipitin test when the mammalian-negative is excluded (97.8 per cent of 1408) or included (77.0 per cent). The major difference is the feeding on horse which the crystallization test showed over twice the percentage expressed in the precipitin test results. Quite possibly the number of mosquitoes which did not react in the Hb test were sufficient to bias the

Table 5-3. Host feeding of Anopheles freeborni collected in the Sacramento Valley of California from June-September 1973 as determined by the precipitin test and the hemoglobin crystallization method (incomplete).

	Test Method	
	Precipitin	Hb Crystallization
No. of mosquitoes tested	1408	860
No. of mosquitoes reacted	1377	444
Host	percent	
Dog	11.1	7.0
Horse	31.0	78.6
Bovine	34.9	11.9
Rabbit	1.6	0.5
Other	0.1	1.1
Negative	21.3	0.0

1 Mammalian positive, but negative to more specific antisera.

percentages towards the positive hosts. If the same percentage of Hb horse positive was calculated on the basis of the number of mosquitoes tested (860) rather than reacted (444), calculation would be more compatible to that of the precipitin test (1377 of 1488 = 97.8) and results would not be as different as expressed in Table 5-3. Conversely, the 21.3% in mammalian-negative category (Table 5-3) might have been horse positives which did not react to horse antisera, and if so, would make the two testing methods more comparable. If the heavy feeding pattern on horse is characteristic primarily of the early and mid-summer, then the completion of the Hb test samples will alter the final percentage of the Hb results considerably.

The proportion of bovine positives is substantially greater by the precipitin test than the Hb test. There is a suspicion that the results reflect the difficulty of processing cow blood meals as noted in Experiment 1 and earlier sections in this experiment.

Further discussion appears inappropriate until all testing is completed.

EXPERIMENT 6: CATALOGUE OF HEMOGLOBIN CRYSTALS

Although work on the catalogue was initiated under NIH support, this work was continued with concurrent support from USA DAMD. Reference Hb crystals were prepared from blood samples of a wide variety of animals that serve as potential blood hosts to blood sucking arthropods. In almost all instances where Hb crystals were prepared, morphologic differences were sufficient to permit ready separation of one animal from another. With some exceptions, many phylogenetically related groups of animals studied have shown morphologic similarity as well as distinctiness in their crystal pattern so that an identification key for host blood meal determinations within a given geographic area is possible.

Photomicrographs of the crystals are catalogued according to host phylogeny and/or Hb crystal morphology. The catalogue can serve as a basis for a dichotomous key to identify unknown blood meals of certain medically important arthropods in a given geographic area. Crystal morphology was sufficiently distinct under light microscopy to eliminate the need for scanning electron micrographs.

Hb crystals were induced from 93% of the mammalian species tested. 89.5% of the mammals tested formed crystals with the normal reagent (0.035M oxalate pH 6.8, 0.025M buffer), the remaining 3.5% were crystallized by other reagents (e.g., 0.21M oxalate and 70% ethanol).

Avian Hb crystallization was considerably less. Only 46.7% of the 45 different bird species tested produced crystals; 22% were induced by the 0.035M reagent, 8% by 70-95% ethanol. Three of thirteen reptiles tested formed Hb crystals with the normal reagent.

The following animals did not form blood crystals with any of the reagents used: six primates including black and white lemur, guatemalan howler monkey, mangabey, mandrill, Celebes crested macaque and kikuyu colobus monkey; two procyonidae including racoon and ringtail; two felids including palestine jungle cat and bobcat and one bovid, the guar.

Overall differences were found to exist between phylogenetic groups in general crystal morphology and speed of crystal formation, the latter was governed by solubility. Crystallization of the different phylogenetic groups was in order of increasing difficulty, as follows: Canidae, Perrissodactyl, Paeunungulata*, Edentata*, Rodentia, Marsupial, Felidae, Artiodactyla, Primates, Aves and Reptilia.

The major accomplishment has been the ready separation of blood samples from several animal groups (i.e., Rodentia) that were either difficult or cumbersome to process by conventional serological means.

One of the major difficulties reported on in the past was our inability to produce crystals consistently from primate blood samples including man. This difficulty has been overcome for the Hb lysates by utilizing the improved crystallizing reagent and procedures.

Needle shaped crystals were found in all phylogenetic groups and may sometimes be a part of crystal avalanching. Marsupials also form tubular crystals. Multiple crystal forms may be observed in blood samples from many Primates. In man, for example, (app.15 [c,d,e,f]) three basic shapes have been found in one blood sample; fine needles, tetradehral and rectangular plates. This variety in external morphologies may depend upon changes in local environmental conditions, e.g., salt and pH and also the oxidation state of the heme iron. The frequency with which Hb polymorphism occurs in Primates may also constitute another reason for multiple crystal form in this group. The Lemurs

and new world monkeys often formed star shaped groups of needles or tabular crystals. Rectangular plates and tetrahedra were common among the old world monkeys.

Hexagonal crystals were often characteristic for rodents especially Sciuromorpha and Myomorpha (app. 16-25). The porcupine formed pentagonal tabloid Hb crystals, whereas guinea pig's formed as symmetrical pyramids (app. 26). Prismatic needles were often diagnostic for Canidae (app. 28, 29, 30 a,b,c). The only member of the paenungulata tested, the hyrax (app. 41) exhibited bipyramid crystals. In general, the perrisodactyls (app. 39 and 40) form monoclinic plates whereas the Artiodactyls produce mainly needles (app. 42-56).

Distinction of the crystal forms prevailed at the generic level in some animal groups, e.g., Primate: Macacca; Rodentia: Dipodomys; and Canidae: Canis. Further detailed studies with Hb solubility may lead to the eventual separation of these genera to some of the individual species. Two sheep breeds, suffolk or suffolk cross (app. 54 c,d,e and 55 a,b) and corriedale (app. 54 f), were separated on crystal form from each other.

Occasional similarities between unrelated species were found. For example, roan antelope crystals (app. 50 b) were hexagonal and like those of California ground squirrel (app. 16 c,d). The antelope's crystals were distinguishable by their heavy etching. The Indian Chinese sika (app. 44 d) and the cape hyrax (app. 41) both form bipyramid Hb crystals which were easily separated by their development. Hyrax Hb formed many crystals very rapidly in contrast to the slow development of only a few sika Hb crystals.

Horse (app. 39) and opossum Hb crystals were also similar in form and time of formation and were separated by the alignment of the crystals in the ring and at the slide edge.

In conclusion, a final working reference catalogue should be constructed taking into account not only crystal form and arrangement, but also development time. Limits on geographic distribution of the vertebrate host will also reduce some of the morphological overlaps.

Table 6-1. Catalogue of animal species tested for their ability to form Hb crystals.

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
MARSUPIALS						
Didelphidae						
Didelphis marsupialis (opossum)	+	+	+	+		4
Macropodidae						
Dendrolagus matsechi (Matschie's tree kangaroo)						
D. goodfellowi goodfellowi (Goodfellow's tree kangaroo)	+					1
Protomnodon agilis jardini (Agile wallaby)	+	+				1
Wallabia bicolor (swamp wallaby)	-	-	+	+		1
Macropus bernardus	+	+				1
Phascolarctidae						
Phascolarctos cinereus (koala bear)	+	+				1
Thylogale parma (white throated wallaby)	-	-	-	-	-	1
Dasyuroidae						
Sarcophilus harrisi (Tasmanian devil)	+	+				3
EDENTATA						
Dasypodidae						
Dasypus novemcinctus (nine-banded armadillo)	+					1
PRIMATES						
Lemuridae						
Lemur variegatus (black & white ruffed lemur)	-	-			-	1
L. fulvus albifrons (white fronted lemur)	+	+	=			2
L. macaco (black lemur)	+	+	+	-		1
Varecia variegatus ruber (red ruffed lemur)	-	-	-	_		2

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Cebidae						
Alouetta villosa (Guatemalan howler monkey)	-	-	-	-	-	1
Ateles sp. (spider monkey)	+	+				1
Cacajao calvus (white uakari)	+	+	+	-		2
C. rubicundus (red uakari)	+	+				3
Callicebus torquatus torquatus (tanhanded titi)	+	+				1
Cercopithecidae						
Macaca arctoides (stump tailed macque)	+	+	+			5
M. mulatta (Rhesus macaque)	+	+	-	-		6
M. fasckularis (crab-eating macaque)	+	+	-			1
M. cynomolgus (Cynomolgus monkey)	+	+				1
M. sylvanus (Barbary ape)	+	+				2
M. nemistrina (pig-tailed macaque)	-	+	-	-		1
M. silenus (lion-tailed macaque)	-	-	-	-		2
Papio anibis (baboon)	+	+				1
Comopithecus hamadryas (Hamadryas baboon)	+	+				2
Erythrocebus patas patas (red patas monkey)	-	+				1
Cynopithecus niger (Celebes crested macaque)	-	-	-	-	-	1
Cercopithecus nictitans (spot nosed guenon)	+	+				2
C. aethiops (green monkey)	+	+				2
Cercocebus sp. (mangabey)	-	-		-	-	1
Theropithecus gelada (gelada baboon)	+	-	+	-		1
Mandrillus sphinx (Mandrill)	-	-				1
Presbytis johnii (Nilgiri langur)	+	+				1
P. obscurus obscurus (spectated langur)	+	+				2

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
P. senex (purple faced langur)	+	+				1
P. entellus (Hanuman's langur)	+	+				1
P. melophus nobilis (brown langur)	+	+	+			1
Pygathrix nemaeus nemaeus (Douc langur)	+	+	+	+		3
Colobus polykomos kikuyuensis (Kikuyu colobus monkey)	-	-	-	-	-	2
Nasalis larvatus orientalis (proboscis monkey)	+	+				1
Hylobatidae						
Hylobates concolor gabriellae (Gabriella crested gibbon)	+	+				1
Pongidae						
Pongo pygmaeus pygameus (orangutan)	+	+				1
P. p. pygmaeus x p. p. abeli	+	+				1
Pan paniscus (pigmy chimpanzee)	-	+				3
P. troglodytes verus (western chimpanzee)	+	+				1
Hominidae						
Homo sapiens (man)	+	+	-	-		17
RODENTIA						
Sciuromorpha						
Sciuridae						
Sciurus griseus (gray squirrel)	+	+	-			9
Eutamias sp. (chipmunk)	+	+	+			
Eutamias amoenus (yellow pine chipmunk)	-	+				1
E. quadrimaculatus (long eared chipmunk)	+					1
Spermophilus beecheyi (Californian ground squirrel)	+	+	+	-		2
S. beldingi (Belding ground squirrel	+	+				3

	pH 6.8 Oxalate 0.035M	0.07M	ETOH	Other	Negative	Number individuals tested
S. lateralis (golden-mantled squirrel)	+					4
S. townsendi (Townsend ground squirrel)	+	+	+			2
Thomomys bottae bottae (gopher)	+		-	-		4
Pedetidae						
Pedestes cafer (springhaas)	+	+	+	+		2
Myomorpha						
Heteromyidae						
Dipodomys ordi (Ord kangaroo rat	+	+				1
D. merriami (Merriam kangaroo rat)	+					1
D. microps (Great basin kangaroo rat)	+	+				2
D. panamintinus (Panamint kangaroo (rat))	+	+				1
D. Heermanni (Heermann kangaroo rat)	+	+				2
Cricetidae						
Peromyscus truei (Pinon mouse)	+	+	-	-		20
P. californicus (California mouse)	+	+				1
P. crinitis (canyon mouse)	+	+				1
P. maniculatus (deer mouse)	+	+				15
P. boylei (brushmouse)	+	+				2
Neotoma lepida (desert woodrat)	+	+				4
N. cinerea (bushytail woodrat)	+	+				2
N. fuscipes (Dusky-footed woodrat)	+	+				4
Sigmodon hispidius (cotton rat)	+	+				1
Microtus longicaudis (vole)	+	+	-			3

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Reithrodontomys megalotis (harvest mouse)	+	+	-			1
Oryzomys palustris (rice rat)	+	+				1
Muridae						
Mus musculus (house mouse)	+	+				6
Rattus rattus (roof rat)	+	+				2
R. norvegicus (Norway rat)	+	+	+			9
Hystricomorpha						
Erethizoa dorsatum (porcupine)	+	+				1
Caviidae						
Cavia porcellus (guinea pig)	+	+				4
Lagomorpha						
Lepus sp. (jackrabbit)	+	+		+		19
Sylvilagus auduboni (desert cottontail)	+	+	-			3
CARNIVORA						
Canidae						
Canis latrans (coyote)	+	+				2
C. familiaris (dog)	+	+				9
C. lupes (wolf)	+	+				1
C. lupus f. familiaris (dingo)	+	+				1
C. aureus syriacus (golden jackal)	+	+	+			3
Chrysocyon brachyurus (named wolf)	+	+				1
Urocyon cinereoargentus californicus (California gray fox)	+	+	+			7
Speothos venaticus venaticus (bush dog)	+	+				1
Procyonidae						
Procyon lotor (raccoon)	-	-	-	-	-	51
Bassariscus astutus (ringtail)	-	-	-		-	1
Ursidae						
Tremarctos ornatus (spectacled bear)	-	+		+		1
Euarctos americanus (blue bear)	+	+				1

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Mustelidae						
Mustela putorius (ferret)	+	+		+		1
Mephitus mephitus (striped skunk)	+	+	+			8
Pinnipedia						
Arctocephalus sp. (cape fur seal)	+	+				4
Callorhinus ursinus (fur seal)	+	+	+			1
Mirounga angustirostris (northern elephant seal)	+	+				1
Zalophus californicus californicus (California sea lion)	+	+				1
Felidae						
Felis domesticus (cat)	+	+				6
F. chauc furax (Palestine jungle cat)	-	-			-	1
F. concolor (mountain lion)	+	+				1
F. silvestris caudata (Russian steppecat)	+	+				1
F. serval ingridi (serval cat)	+	+				1
F. lynx (bobcat)	-	-	-	-		2
Panthera leo (African lion)	+	+				1
P. tigris tigris (Bengal tiger)	+	+				3
P. tigris altaica (Siberian tiger)	+	+				4
P. onca (jaguar)	+	+				3
P. onca (black phase)	+	+				1
P. pardus japonesis (North Chinese leopard)	+	+				1
P. pardus delacouri (Indo-Chinese leopard)	+	+				2
Neofelis nebulosa (clouded leopard)	+	-	+	-		1
Acinonyx jubatus (cheetah)	+	+				3
Genetta genetta felina		+				1
CETACEA						
Tursiops gilli (bottle-nosed dolphin)	+	+	+	+		1
T. truncatus (bottle-nosed dolphin)	+	+	+	+		1

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
HYRACOIDAE						
Procaviidae						
Procavia capensis capensis (South African rock hyrax)	+	+				2
PERISSODACTYLA						
Equidae						
Equus caballus (horse)	+	+	+	+		21
E. przewalski przewalski (Przewalski's horse)	+	+				2
E. zebra hartmani (Hartmann's mountain zebra)	+	+				1
Asinus africanus f. asinus (Sicilian donkey)	+					1
Rhinocerotidea						
Ceratotherium simum simum (Southern white rhinoceros)	+	+				1
Rhinoceros unicornis (great Indian rhinoceros)	+	+				1
Tapiroidea						
Tapirus sp. (tapir)	+	+	+	-		1
T. bairdii (Baird's tapir)	+	+				1
ARTIODACTYLA						
Suiformes						
Suidae						
Sus scrofa (pig)	+	-				12
Tayassuidae						
Tayassu tajacu sonoriensis (colared peccary)	+	+				1
Hippopotamidae						
Choeropsis liberiensis (pigmy hippopotamus)	+	+	+			1
Tylopoda						
Camelidae						
Camelus ferus f. dromedarius (dromedary)	-	+	-			2

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Lama glama glama (llama)	+	+	+			
L. guanacoe guanacoe (guanaco)	+	+				1
L. guanacoe f. glama (guanaco)	+	+				1
Ruminantia						
Cervidae						
Odocoileus hemionus (mule deer)	+	+				2
Rangifer tarandus (reindeer)	+	+				4
Muntiacus reevesi reevesi (Reeves muntjac)	+	+				2
Cervus elaphus sibiricus (Altai wapiti)	+	+				2
C. nipon pseudaxis (Indian-Chinese sika)	+	+				1
Antilocapriadae						
Antilocapra americana americana (pronghorn)	+	+				2
Bovidae						
Bos gaurus gaurus (gaur)	-	-			-	1
Bos taurus (jersey, hereford, guernsey, shorthorn, Charolais, angus, holstein and Brangus)	+	+	-			107
Beefalo (13/16 cow, 3/16 bison)	+	+	-			1
Bison bison bison (bison)	+	+				1
B. b. bonasus (newborn Wisent)	+	+				1
Hippotragus niger niger (Sable antelope	+	+				1
H. equinus cottoni (Angolan roan antelope)	+	+	+			1
Oryx leucoryx (Arabian oryx)	+	+				1
Connochaetes taurinus albojubatus (Eastern white-bearded gnu)	+	+				2
C. gnou (white tailed gnu)	+	+				1
Tragelaphus spekii spekii (East African sitatunga)	+	+				2
Taurotragus oryx pattersoni X Tragelaphus s. strepsiceros (Eland x kudu)	+	+				1
Boselaphus tragomacellus (Nilgai)	+	+				2

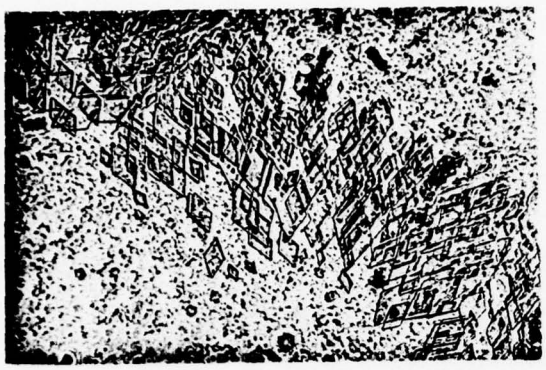
	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Addax nasomaculatis (Addax)	+	+				1
Aepyceros melampus rendilus	+	+				1
Antidorcas marsupialis marsupialis (Springbok)	+	+				1
Antilope cervicapra (Indian black buck)	+	+				1
Damaliscus dorcas dorcas (Bontebok)	+	+	+	+		2
D. dorcas phillipsi X D. dorcas dorcas (Bontebok X Blesbok)	+	+	+	+		1
Gazella dama dama (Dama gazelle)	+	+				1
G. granti roosevelti (Grant's gazelle)	+	+	+	+		1
Cephalophus silvicultor silvicultor (yellow backed duiker)	+	+				1
Ovis aries (domestic sheep) (corriedale)	+	+				6
(suffold)	+	+				6
Ovis ammon musimon (mouflon)		+	-	-		2
O. a. f. aries (four horned sheep)	+	+				1
Capra aegagrus f. hircus (pigmy Mexican hair, La Mancha goat, Nubian)	+	+				11
C. a. cretila (Cretian goat)	+	+				1
C. falconeri heptneri (Turkomen markhor)	+	+				1
C. iben severtzovi (newborn West cave tur)	+	+				1
Hemitragus jemlahicus (Himilayan tur)	+	+				1
AVES						
Rheidae						
Pternocnemia pennata pennata (Darwin's rhea)	+	+				1
Anseriformes						
Anas acuta (pintail)	-	-			-	2
A. flavirostris flavirostris chilean Teal)	-	-	-		-	1

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
A. platyrhnchos (mallard)	-	-			-	4
A. platyrhnchos wyvilliana (Hawaiian duck)	-	-	-		-	1
Anser brachyrhynchus (pink footed goose)	+		+			1
A. coerulescens (snow goose)	-	+	+			1
Aythya valisineria (canvas back duck	-	-	-		-	4
Branta leucopsis (claris goose)	-	-	+			1
Falconiformes						
Elanus leucurus (white-tailed kite)			+			1
Accipiter cooperi (coopers hawk)	-	-	-			1
Circus cyaneus (marsh hawk)	-	+	+			1
Buteo jamaicensis (red-tailed hawk)	-	-	-	-	-	6
B. lineatus (red-shouldered hawk)	-	-	-	-	-	2
B. swainsoni (Swainson's hawk)	-	-	-	-	-	1
Aguila chrysaetos (golden eagle)	-	-	-		-	1
Morphus guianensis (guinean eagle)	-	-	+			1
Falco sparvericus (kestral)	+	+		+		3
Polemaetus bellicosus (Martial eagle)	-	-	-	-		1
Galliformes						
Chicken	-	-	-	+		11
Bantu chicken	-	+	-			1
Meleagris gallopavo (turkey)	+	+		-		5
Quail	-	-		+		1
Guinea fowl	+	+				2
Colinus virginianus (Bobwhite)	-	-		+		2
Phasianus colchicus (Ringneck)	+	+	-	-		6
Chrysolophus pictus (golden)	+	+				1
Lophura swinhoeii (Swinhoes pheasant)		-		+		1
Columbiformes						
Columbia livia (pigeon)	+	+	+			4

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Stringiformes						
Otus asio (screech owl)	-	-			-	1
Bubo virginianus (great horned owl)	-	-		-	-	3
Tyto alba (Barn owl)			+			1
Piciformes						
Colaptes cafer (Red shafter flicker)		+		+		2
Passeriformes						
Corvidae						
Corvus brachyrhnchos (common crow)		+	+			1
Aphelocoma coerulescens (scrub jay)	-	-	-		-	4
Mimidae						
Mimus polyglottos (mocking bird)	-	-	-		-	2
Turdidae						
Turdus migratorius (Robin)	+	+	+	+		6
Hylocichla ustulata (Swainsons thrush)	-	-	-		-	2
Bombycillidae						
Bombycilla cedrorum (cedar waxwing)	+		-			3
Sturnidae						
Sturnus vulgaris (starling)	-	-	+			10
Ploceidae						
Passer domesticus (house sparrow)	-	-	-		-	8
Icteridae						
Xanthocephalus xanthocephalus (yellow headed blackbird	-	-	-		-	5
Agelaius tricolor (tricolored blackbird)	-	-	-		-	5
A. phoeniceus (red-winged blackbird)	-	-	-	-	-	9
Euphagus cyanocephalus (Brewers blackbird)	+		-			16

	pH 6.8 Oxalate 0.035M	pH 6.8 Oxalate 0.07M	ETOH	Other	Negative	Number individuals tested
Molothrus ater (brownheaded cowbird)	-	-	-		-	3
Icterus bullocki (bullocks oriole)	-	-	-		-	3
Fringillidae						
Carpodacus mexicanus (house-finch)	-	-	-		-	10
Pheucticus melanocephalus (blackheaded grossbeak)	-	-	-		-	1
Pipilo fuscus (brown towhee)	-	-	-		-	1
Chondestes grammacus (lark-sparrow)	-	-	-		-	1
REPTILIA						
Chelydridae						
Chrysomys scripta elegans (red eared turtle)	+	+	+			1
Iguanidae						
Iguana iguana (green iguana	+	+				1
Varanus komodensis (komodo dragon)	-	-	-		-	1
Sceloporus occidentalis (N.W. fence lizard)	-	-	-		-	2
Uta stanoburiana	-	-			-	3
Callisaurus draconoides	-	-			-	1
Phrynosoma cornutum (horned lizard)	-	-	-		-	1
Colubridae						
Natrix rhombifera rhombifera (diamond back water snake)	+					2
Thamnophis proximus (ribbon snake)	+					1
T. elegans (garter snake)		-	-		-	1
Pituophis melanolguous cafenifer (gopher snake)	-				-	1
Lampropeltis getulus californiae (californian king snake)	-				-	1

App. 1: class: MAMMALIA; order: MARSUPIALIA

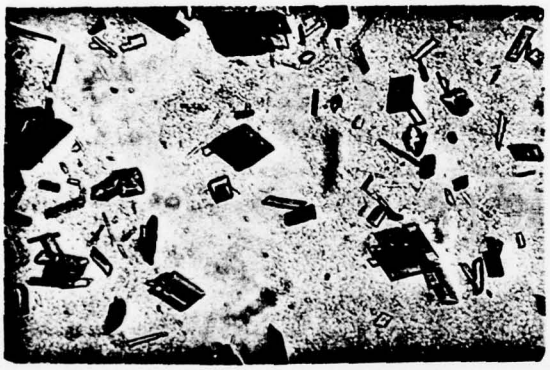


a.) Didelphidae

Didelphis marsupialis (common opossum)

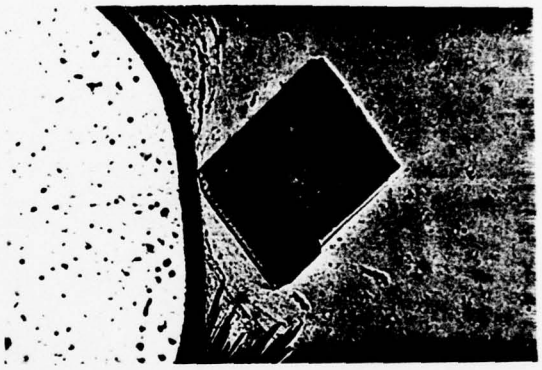
b.) Didelphidae

D. marsupidalis (common opossum)



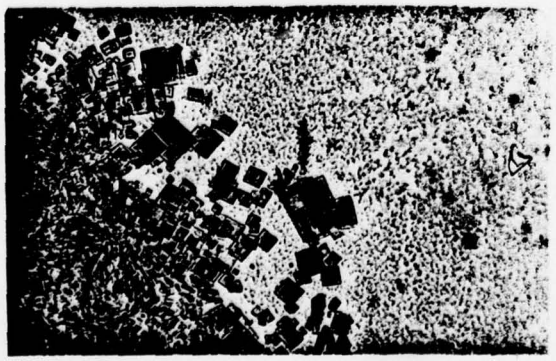
c.) Dasyuridae

Sarcophilus harrisi (Tasmanian devil)



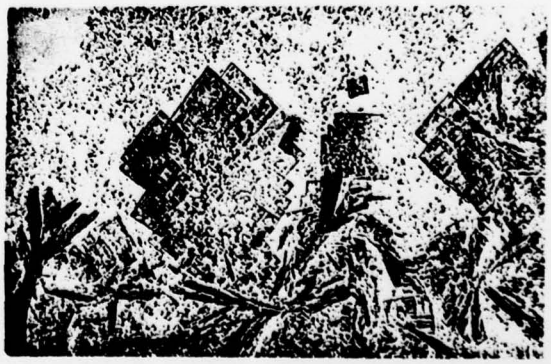
d.) Dasyuridae

S. harrisi (Tasmanian devil)



e.) Phascolarctidae

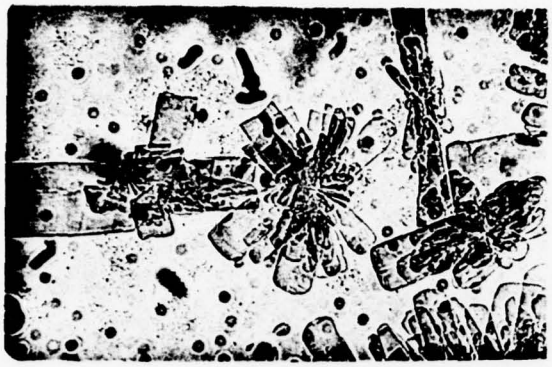
Phascolarctos cinereus
(Koala bear)



f.) Phascolarctidae

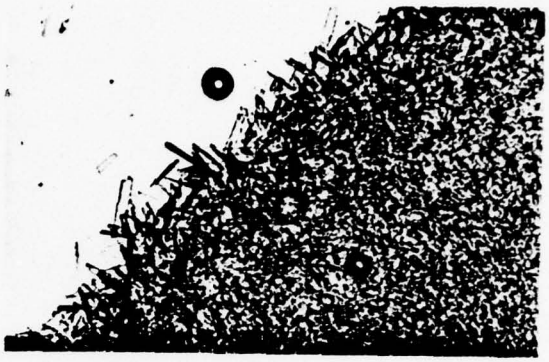
P. cinereus
(Koala bear)

App. 2: class: MAMMALIA; order: MARSUPIALIA



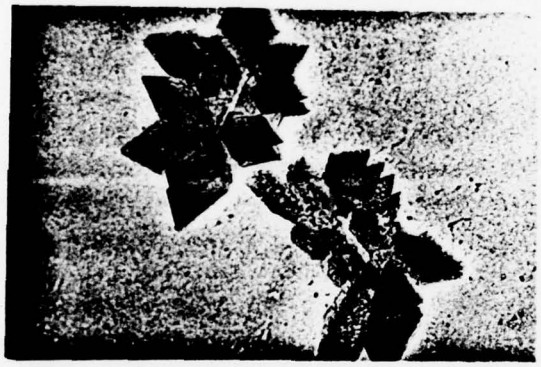
a.) Macropodidae

Dendrolagus matsechi (Matschie's tree kangaroo)



b.) Macropodidae

D. matsechi (Matschie's tree kangaroo)

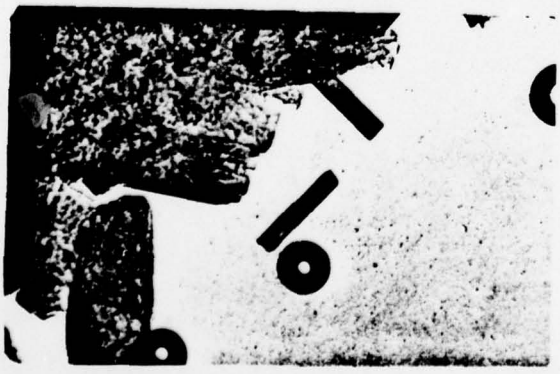


c.) Macropodidae

D. matsechi (Matschie's tree kangaroo)

d.) Macropodidae

D. goodfellowi (Goodfellow's tree kangaroo)



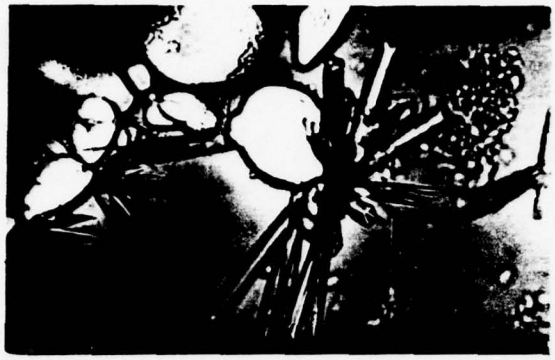
f.) Macropodidae

Protomnodon agilis jardini (Agile wallaby)

g.) Macropodidae

P. agilis jardini (Agile wallaby)

App. 3: class: MAMMALIA; order: MARSUPIALIA



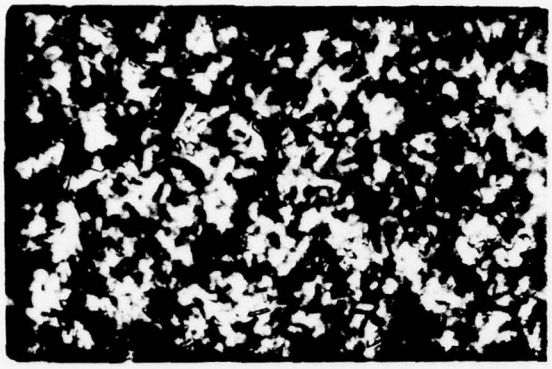
a.) Marcopodidae

Wallabia bicolor
(Swamp wallaby)

b.) Marcopodidae
Macropus bernardus
(Walleroo)

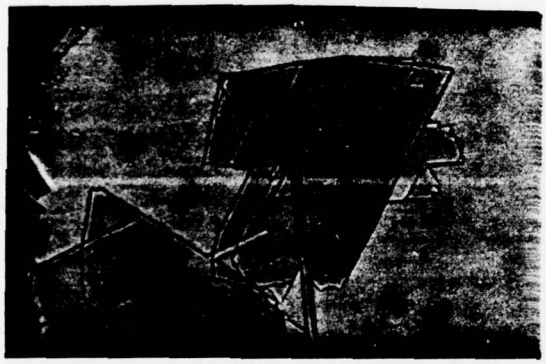
c.) Macropodidae
M. bernardus
(Walleroo)

App. 4: class: MAMMALIA; order: EDENTATA

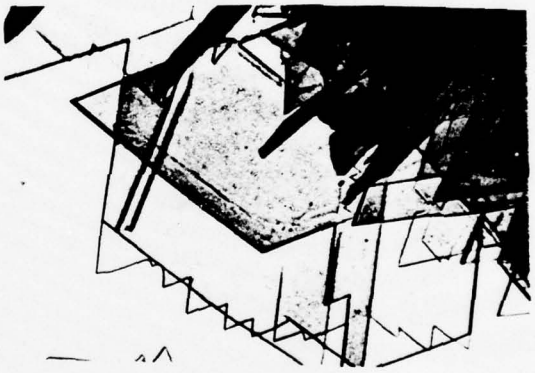


a.) Dasypodidae
Dasypus novemcinctus
(Nine-banded armadillo)

b.) Dasypodidae
D. novemcinctus
(Nine-banded armadillo)

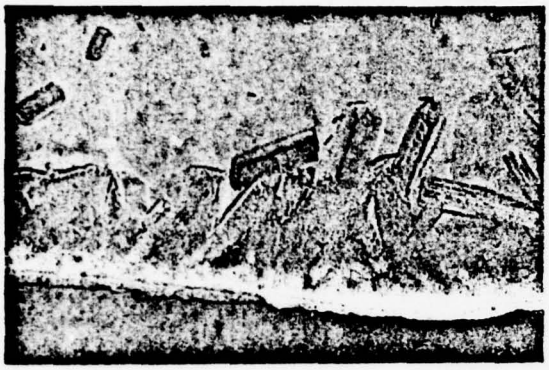


c.) Dasypodidae
D. novemcinctus
(Nine-banded armadillo)



d.) Dasypodidae
D. novemcinctus
(Nine-banded armadillo)

App. 5: class: MAMMALIA; order: PRIMATES

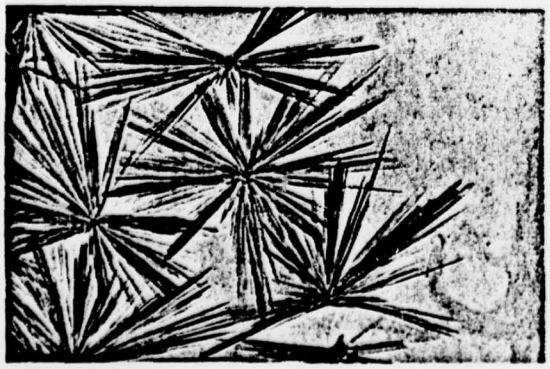


a.) Lemuridae
Lemur macaco
(Brown lemur)

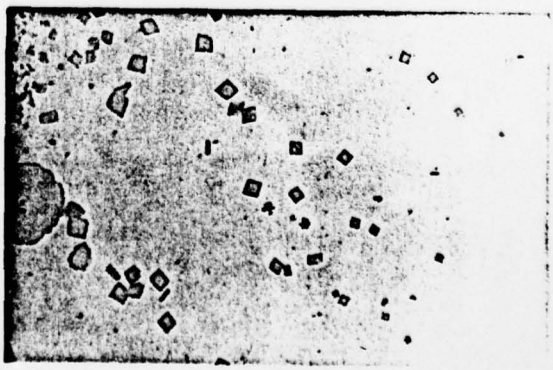
b.) Lemuridae
Lemur macaco
(Black lemur)

c.) Lemuridae
L. macaco
(Black lemur)

d.) Lemuridae
L. variegatus ruber
(Ruffed lemur)

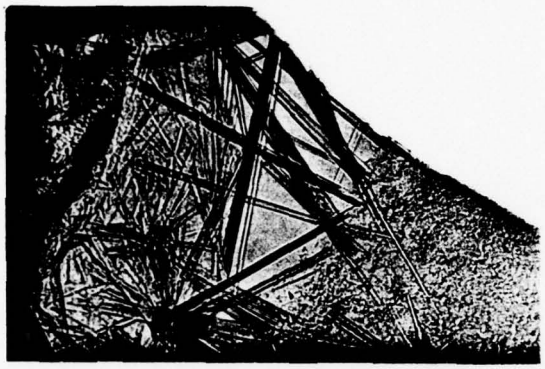


3.) Lemuridae
L. variegatus ruber
(Ruffed lemur)



f.) Lemuridae
L. variegatus ruber
(Ruffed lemur)

App. 6: class: MAMMALIA; order: PRIMATES



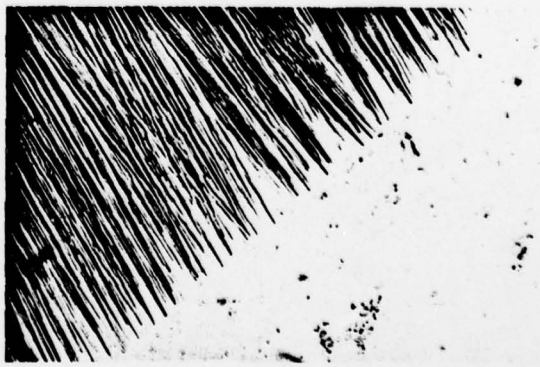
a.) Cebidae
Ateles
(Spider monkey)

b.) Cebidae
Ateles
(Spider monkey)

c.) Cebidae
Cacajao calvus
(Bald uakari)

d.) Cebidae
C. rubicundus
(Red Uakari)

e.) Cebidae
C. rubicundus
(Red uakari)

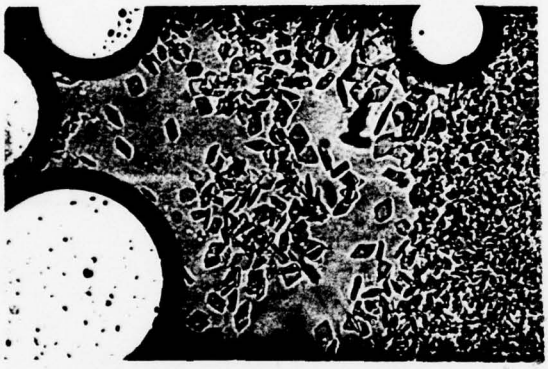


f.) Cebidae
Callicebus torquatus
(tan handed titi)

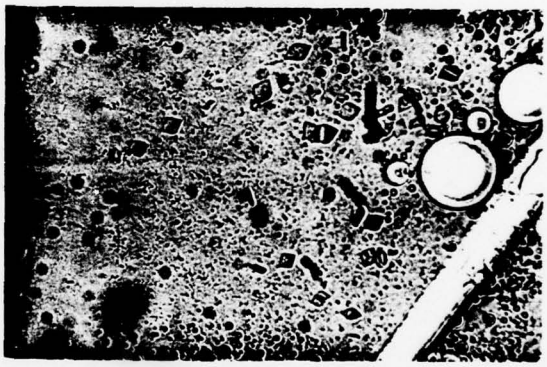
App. 7: class: MAMMALIA; Order: PRIMATES

a.) Cebidae
C. torquatus
(tan handed titi)

b.) Cercopithecidae
Macaca mulatta
(Rhesus macaque)



c.) Cercopithecidae
M. arctoides
(Stump-tailed macaque)

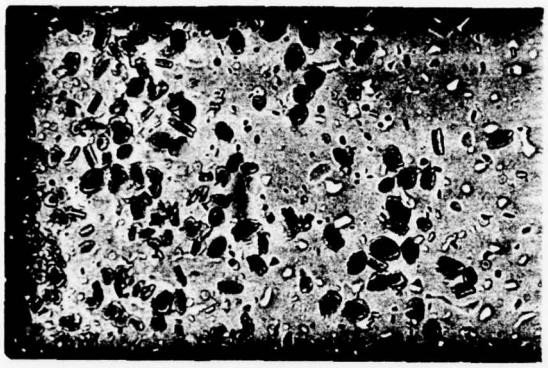


d.) Cercopithecidae
M. fascicularis
(Crab-eating macaque)

e.) Ceropithecidae
M. cynomolgus (Cynomolgus monkey)

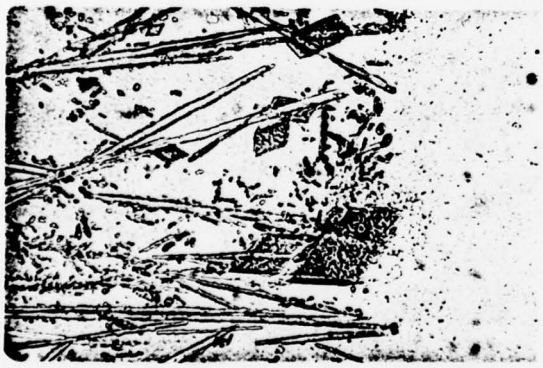
f.) Cercopithecidae
M. nemestrina
(Pig-tailed macaque)

App. 8: class: MAMMALIA; order: PRIMATES



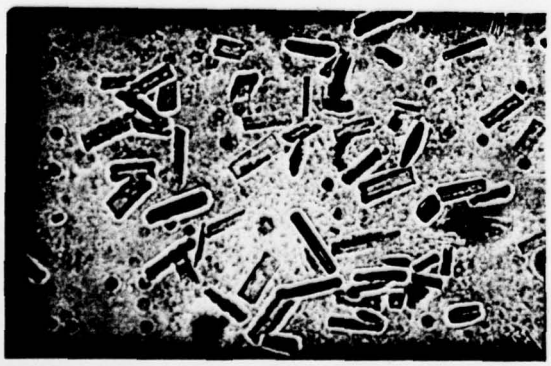
a.) Cercopithecidae
M. nemestrina
(Pig-tailed macaque)

b.) Cercopithecidae
M. silenus
(Lion-tailed macaque)



c. Cercopithecidae
M. sylvanus
(Barbary ape)

d.) Cercopithecidae
M. sylvanus
(Barbary ape)

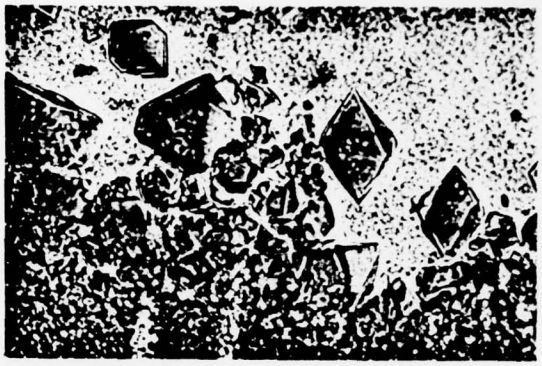


e.) Cercopithecidae
Papio
(Baboon)

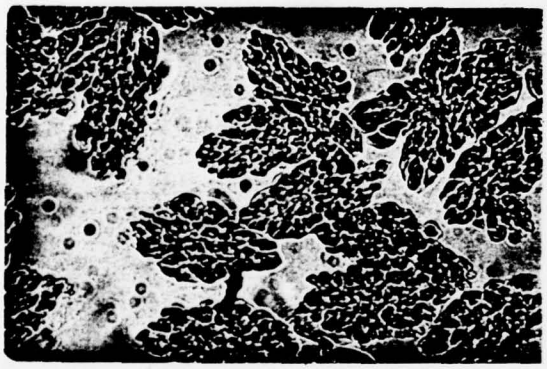
f.) Cercopithecidae
Papio
(Baboon)

App. 9: class: MAMMALIA; order: PRIMATES

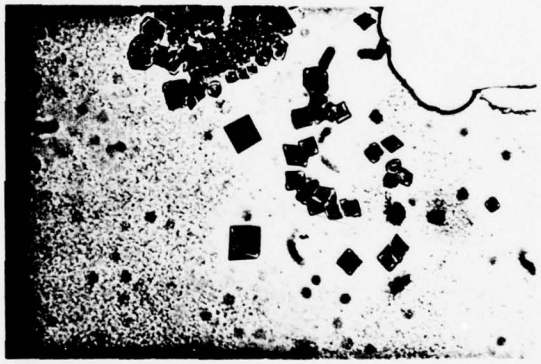
a.) Cercopithecidae
Papio hamadryar
(Hamadryar baboon)



b.) Cercopithecidae
Cercopithecus patas
(Patas monkey)

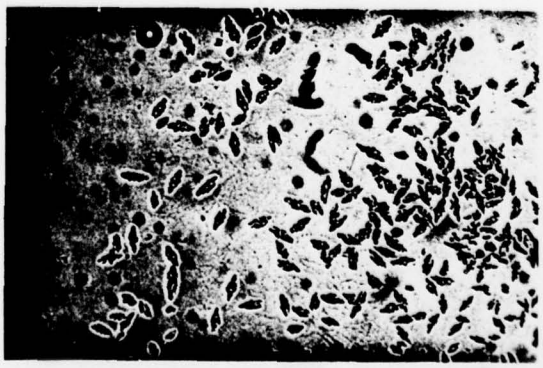


c.) Cercopithecidae
C. petavrista
(Spot-nosed guenon)



d.) Cercopithecidae
C. petavrista
(Spot-nosed guenon)

e.) Cercopithecidae
C. petaurista
(Spot-nosed guenon)



f.) Cercopithecidae
C. aethiops sabeus
(Green monkey)

AD-A052 428 CALIFORNIA UNIV DAVIS DEPT OF ENTOMOLOGY F/G 6/3
IDENTIFICATION OF HOST BLOOD MEALS IN ARTHROPODS.(U)
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UNCLASSIFIED NL

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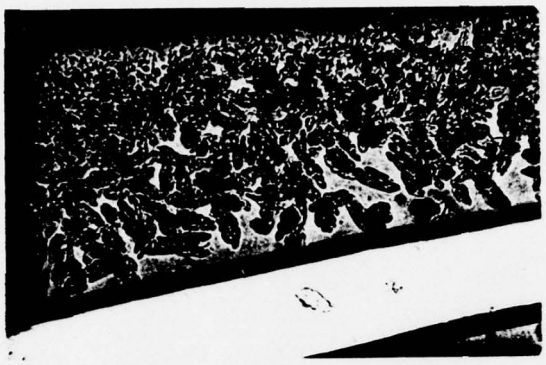
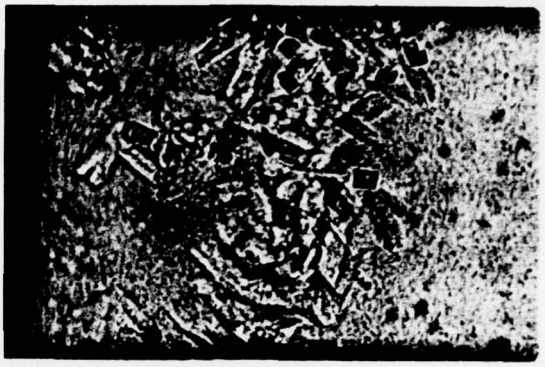
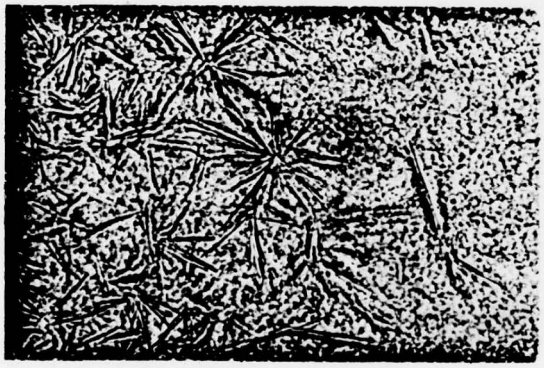
App. 10: class: MAMMALIA; order: PRIMATES



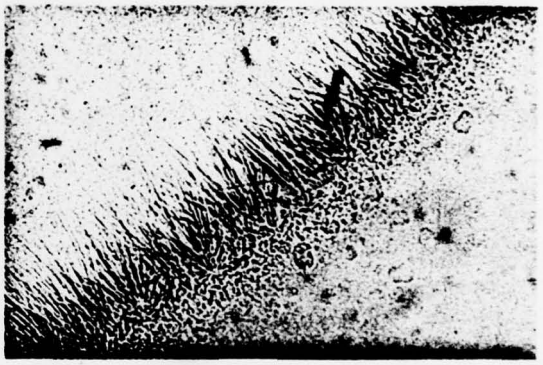
a.) Cercopithecidae
C. aethiops sabeus
(Green monkey)



b.) Cercopithecidae
Theropithecus gelada
(Gelada)

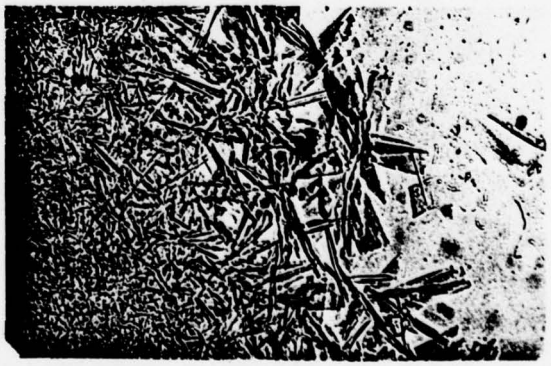


c.) Cercopithecidae
T. gelada
(Gelada)



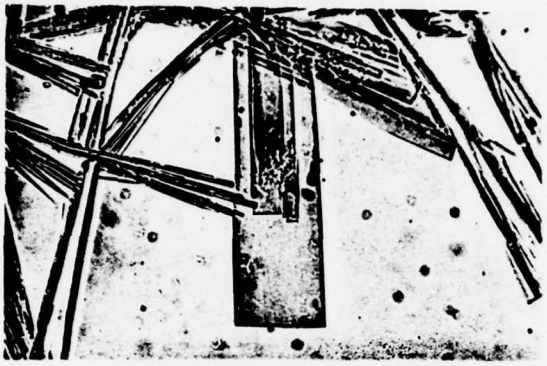
d.) Cercopithecidae
Presbytis johnii
(John's langur)

e.) Cercopithecidae
P. johnii
(John's langur)



f.) Cercopithecidae
P. obscurus obscurus
(Dusky leaf monkey)

App. 11: class: MAMMALIS; order: PRIMATES



a.) Cercopithecidae
P. obscurus obscurus
(Dusky leaf monkey)

b.) Cercopithecidae
P. obscurus obscurus
(Dusky leaf monkey)

c.) Cercopithecidae
P. entellus
(Entellus langur)

d.) Cercopithecidae
P. entellus
(Entellus langur)

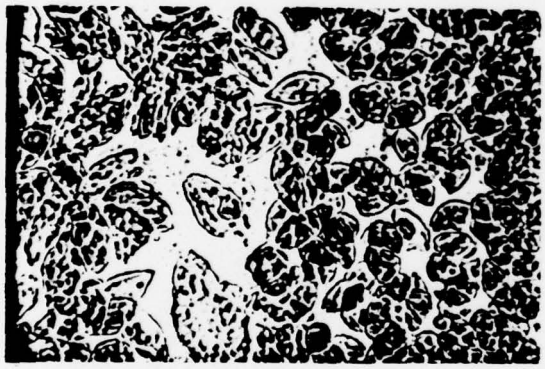
e.) Cercopithecidae
P. melalophus
(Banded leaf monkey)

f.) Cercopithecidae
P. melalophus
(Banded leaf monkey)

App. 12: class: MAMMALIA; order: PRIMATES

a.) Cercopithecidae
P. melalophus
(Banded leaf monkey)

b.) Cercopithecidae
P. melalophus
(Banded leaf monkey)



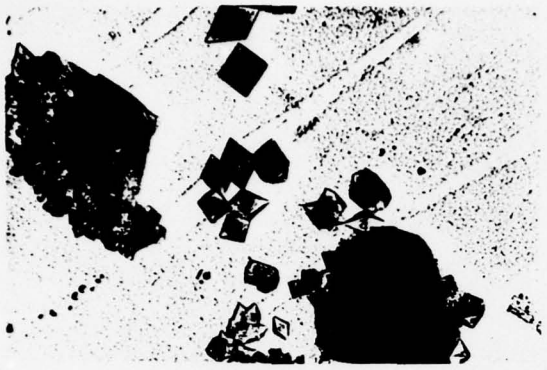
c.) Cercopithecidae
P. senex
(Purple faced langur)

d.) Cercopithecidae
P. senex
(Purple faced langur)

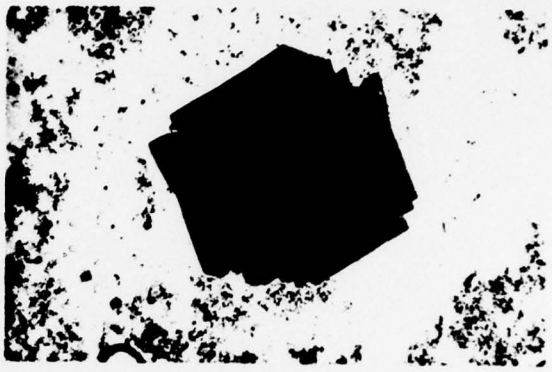
e.) Cercopithecidae
Pygathrix namaeus namaeus
(Douc langur)

f.) Cercopithecidae
P. namaeus namaeus
(Douc langur)

App. 13: class: MAMMALIA; order: PRIMATES



a.) Cercopithecidae
P. nemaeus nemaeus
(Douc langur)



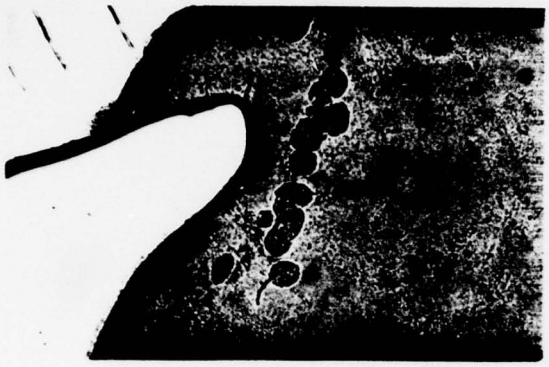
b.) Cercopithecidae
P. nemaeus nemaeus
(Douc langur)

c.) Cercopithecidae
P. namaeus namaeus
(Douc langur)

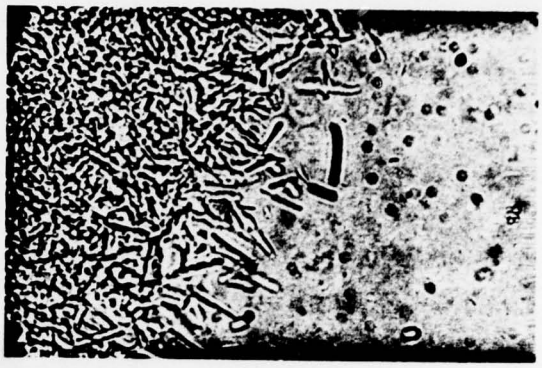
d.) Cercopithecidae
P. namaeus namaeus
(Douc langur)

e.) Cercopithecidae
Nasalis larvatus
(Proboscis monkey)

App. 14: class: MAMMALIA; order: PRIMATES

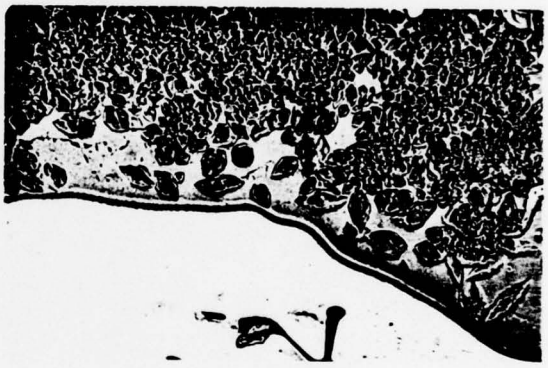


a.) Pongidae
Hylobates concolor gabriellae



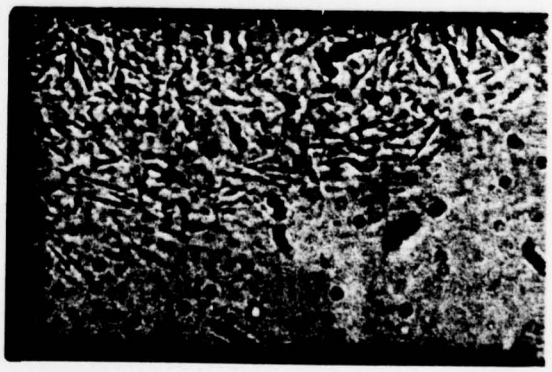
b.) Pongidae
Pongo pygmaeus pygmaeus

c.) Pongidae
P. pygmaeus pygmaeus
(Orang-utan)



d.) Pongidae
P. pygmaeus pygmaeus
(Orang-utan)

e.) Pongidae
P. pygmaeus pygmaeus
(Orang-utan)



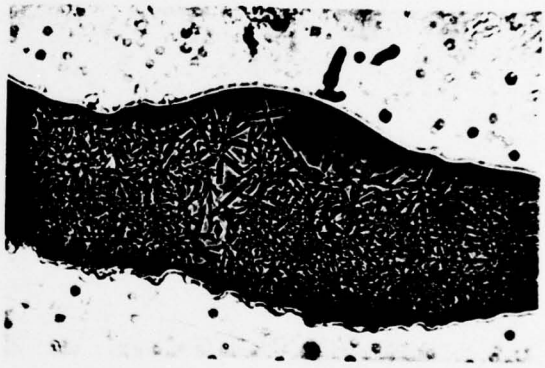
f.) Pongidae
Pan paniscus
(Pygmy chimpanzee)

App. 15: class: MAMMALIA; order: PRIMATES

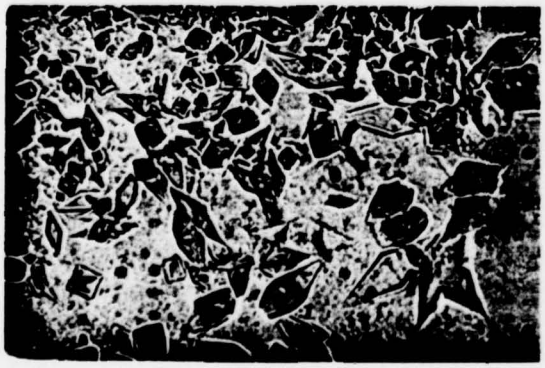
a.) Pongidae
P. paniscus
(Pygmy chimpanzee)

b.) Pongidae
P. troglodytes verus
(Western chimpanzee)

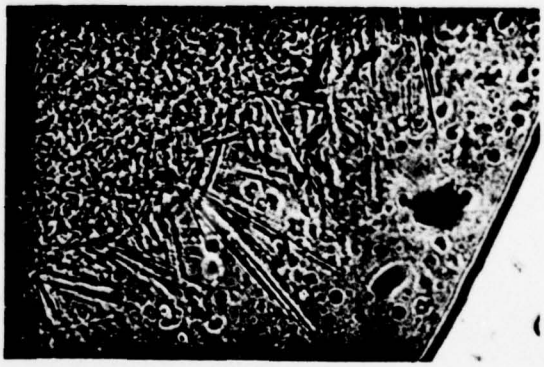
c.) Homindae
Homo sapiens
(Man)



d.) Homindae
H. sapiens
(Man)

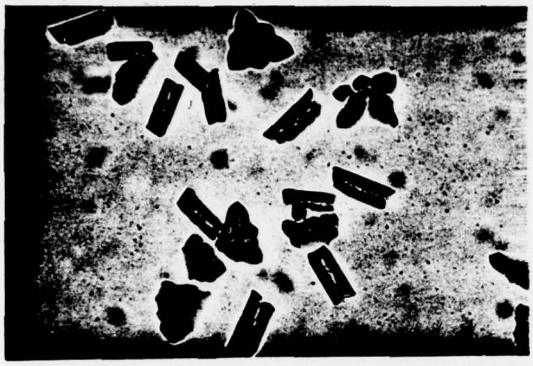


e.) Homindae
H. sapiens
(Man)



f.) Homindae
H. sapiens
(Man)

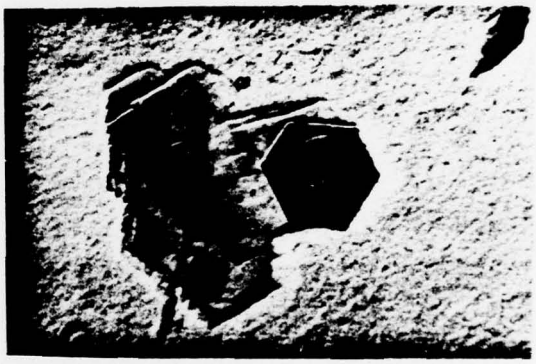
App. 16: class: MAMMALIA; order: RODENTIA



a.) Sciuridae
Sciurus griseus
(Western gray squirrel)

b.) Sciuridae
S. niger/carolinensis
(Gray/fox squirrel)

c.) Sciuridae
Spermophilus beecheyi
(California ground squirrel)



c.) Sciuridae
S. beecheyi
(California ground squirrel)

e.) Sciuridae
S. beldingi
(Beldings ground squirrel)

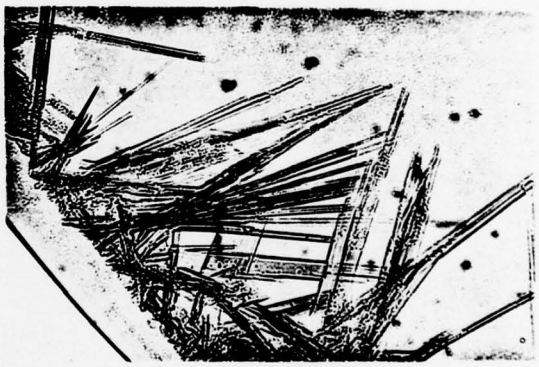
f.) Sciuridae
S. beldingi
(Beldings ground squirrel)

App. 17: class: MAMMALIA; order: RODENTIA

a.) Sciuridae
S. beldingi
(Beldings ground squirrel)

b.) Sciuridae
S. lateralis
(Golden mantled ground squirrel)

c.) Sciuridae
S. lateralis
(Golden mantled ground squirrel)

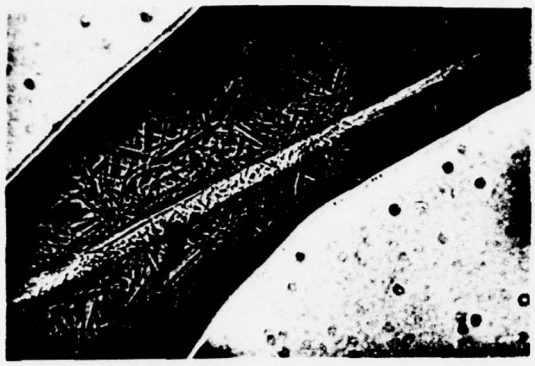


d.) Sciuridae
Eutamias quadrimacuratus
(Long eared chipmunk)

e.) Sciuridae
Spermophilus townsendii
(Townsends ground squirrel)

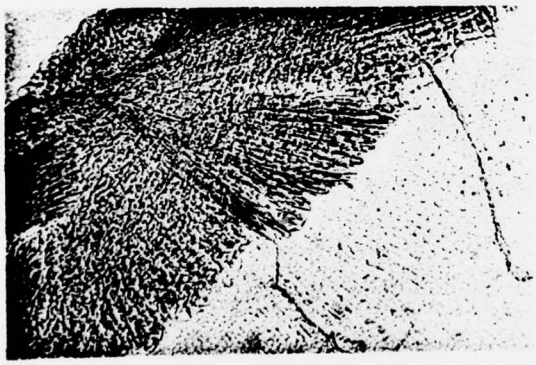
f.) Sciuridae
S. townsendii
(Townsends ground squirrel)

App. 18: class: MAMMALIA; order: RODENTIA

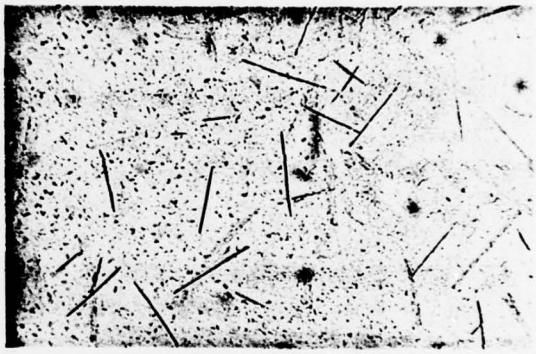


a.) Sciuridae
Eutamias amoenus
(Yellow pine shipmunk)

b.) Sciuridae
E. amoenus
(Yellow pine chipmunk)

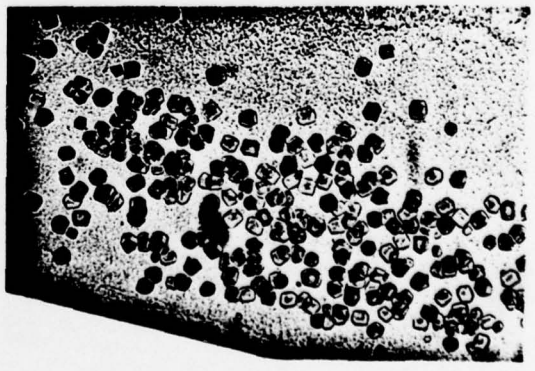


c.) Geomyidae
Thomomys bottae
(Bottar pocket gopher)



d.) Geomyidae
T. bottae
(Bottar pocket gopher)

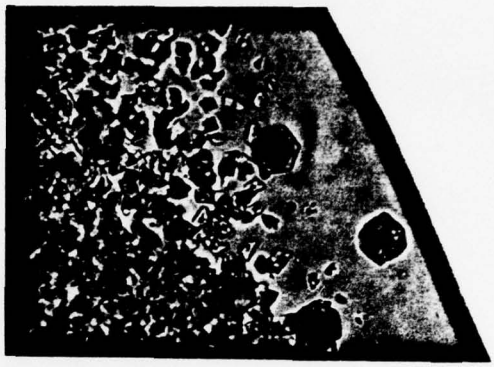
e.) Pedetidae
Pedetes capensis
(Springhaas)



f.) Heteromyidae
Dipodomys merriami
(Merriams kangaroo rat)

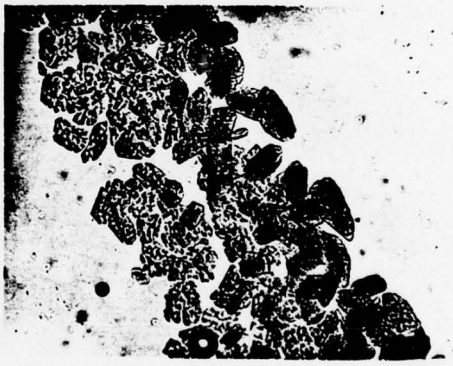
App. 19: class: MAMMALIA; order: RODENTIA

a.) Heteromyidae
D. ordii
(Ord's kangaroo rat)

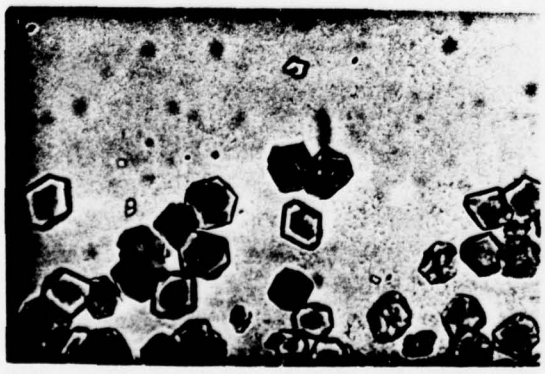


b.) Heteromyidae
D. ordii
(Ord's kangaroo rat)

c.) Heteromyidae
D. heermanni
(Heermann's kangaroo rat)



d.) Heteromyidae
D. heermanni
(Heermann's kangaroo rat)



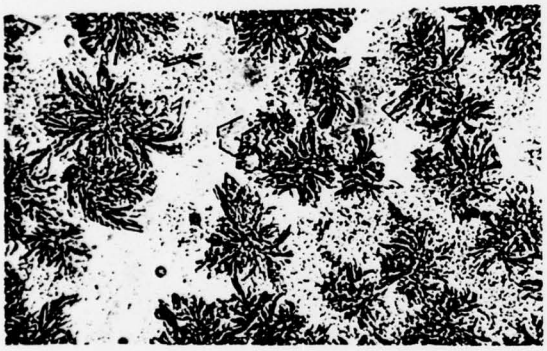
e.) Heteromyidae
D. panamintinus
(Panamint kangaroo rat)

f.) Heteromyidae
D. microps
(Chisel-toothed kangaroo rat)

App. 20: class: MAMMALIN; order: RODENTIA

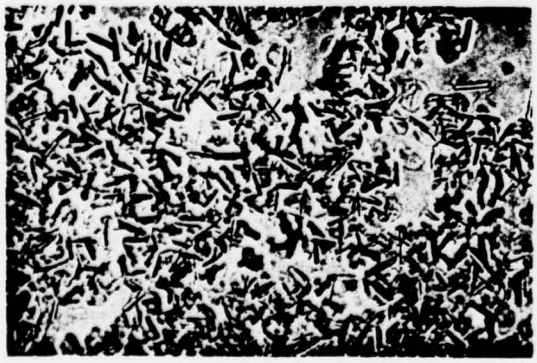
a.) Cricetidae
Reithrodontomys megalotis
(Harvest mouse)

b.) Cricetidae
R. megalotis
(Harvest mouse)

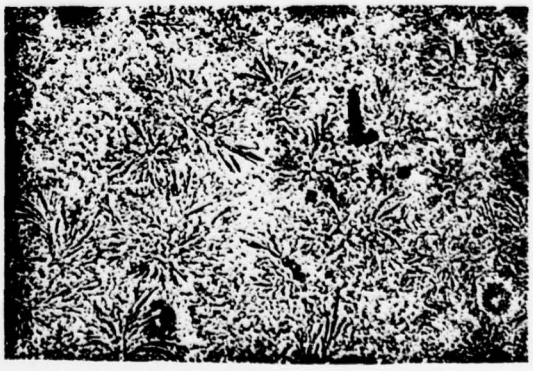


c.) Cricetidae
Peromyscus truei
(Pinon mouse)

d.) Cricetidae
P. truei
(Pinon mouse)

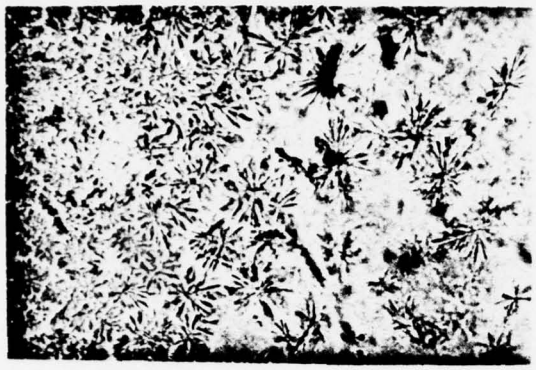


e.) Cricetidae
P. truei
(Pinon mouse)



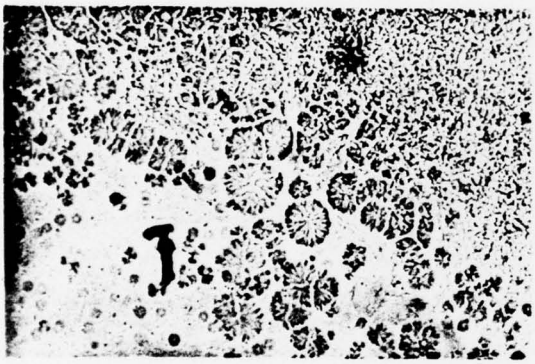
f.) Cricetidae
P. truei
(Pinon mouse)

App. 21: class: MAMMALIA; order: RODENTIA



a.) Cricetidae
P. boyli
(Brush mouse)

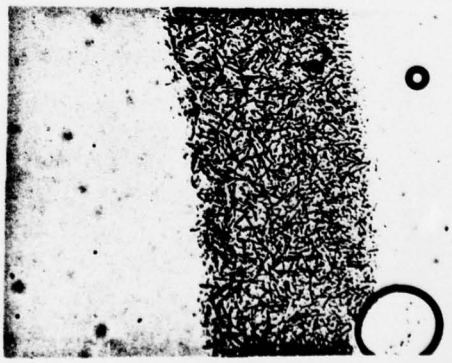
b.) Cricetidae
P. boyli
(Brush mouse)



c.) Cricetidae
P. boyli
(Brush mouse)

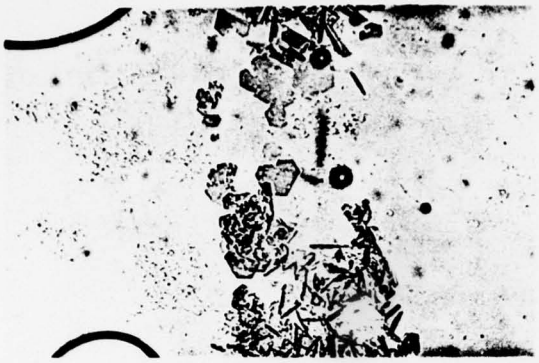
d.) Cricetidae
P. maniculatus
(Deer mouse)

e.) Cricetidae
P. maniculatus
(Deer mouse)

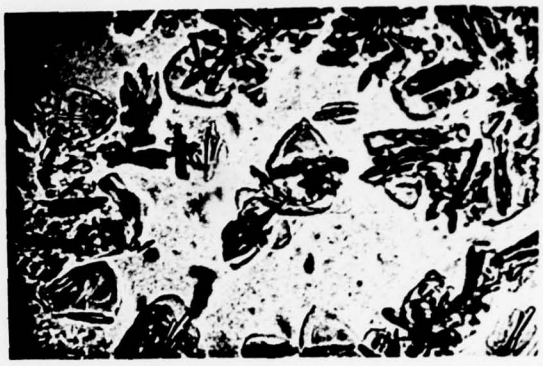


f.) Cricetidae
P. crinitus
(Canyon mouse)

App. 22: class: MAMMALIA; order: RODENTIA



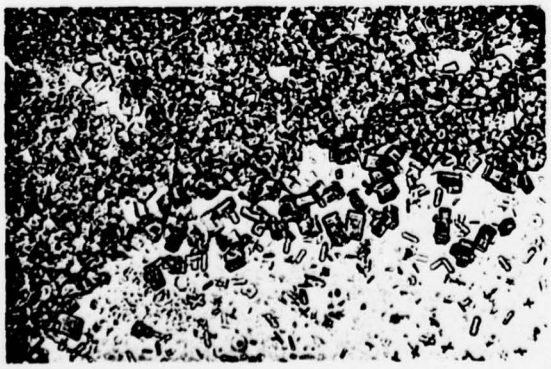
a.) Cricetidae
P. crinitus
(Canyon mouse)



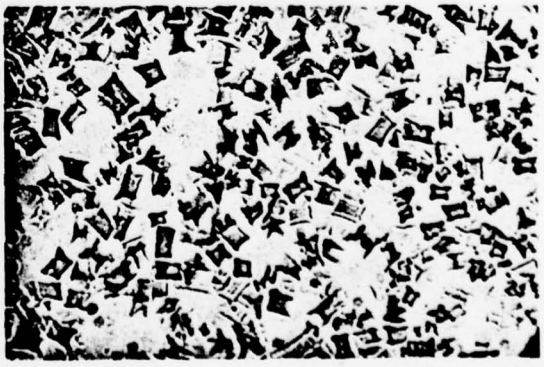
b.) Cricetidae
P. gossypinus
(Cotton mouse)

c.) Cricetidae
P. californicus
(California mouse)

d.) Cricetidae
Sigmodon hispidus
(Hispid cotton rat)

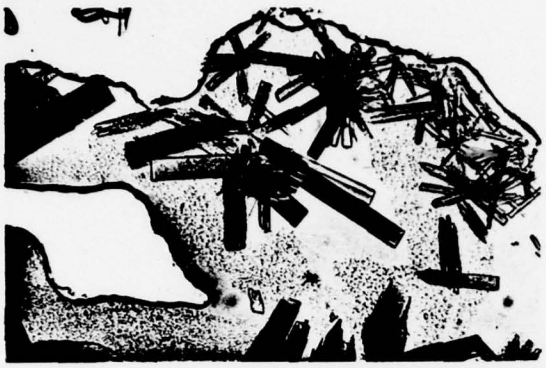


e.) Cricetidae
S. hispidus
(Hispid cotton rat)



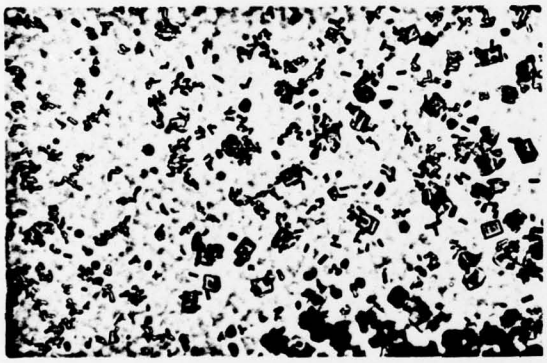
f.) Cricetidae
Neotoma cinerea
(Bushy-tailed wood rat)

App. 23: class: MAMMALIA; order: RODENTIA



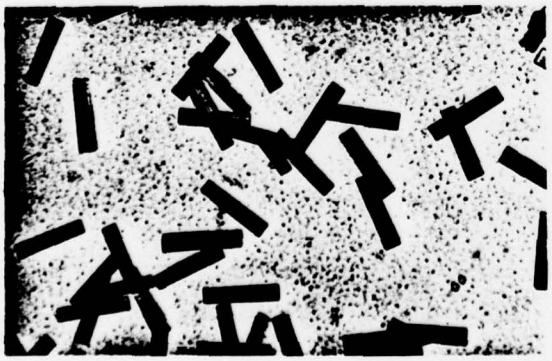
a.) Cricetidae
N. lepida
(Desert wood rat)

b.) Cricetidae
N. lepida
(Desert wood rat)

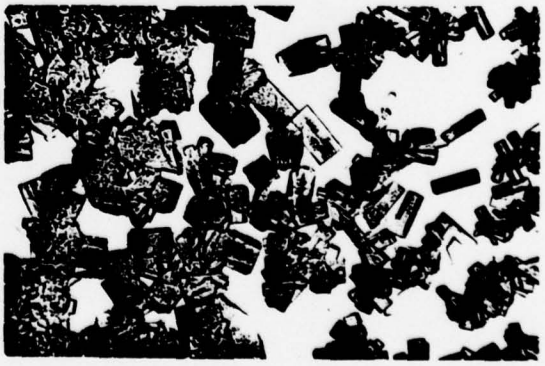


c.) Cricetidae
N. lepida
(Desert wood rat)

d.) Cricetidae
N. lepida
(Desert wood rat)



e.) Cricetidae
N. fuscipes
(Dusky-footed wood rat)

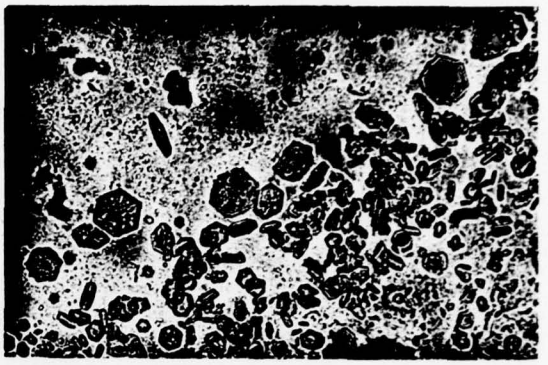


f.) Cricetidae
N. fuscipes
(Dusky footed wood rat)

App. 24: class: MAMMALIA; order: RODENTIA

a.) Cricetidae

N. fuscipes
(Dusky-footed woodrat)

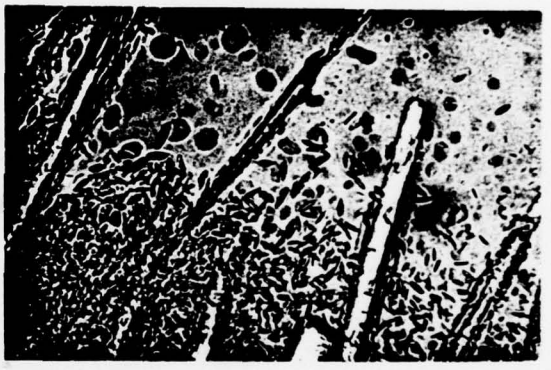


b.) Cricetidae

Microtus longicaudus
(Long-tailed vole)

c.) Muridae

Mus musculus
(House mouse)

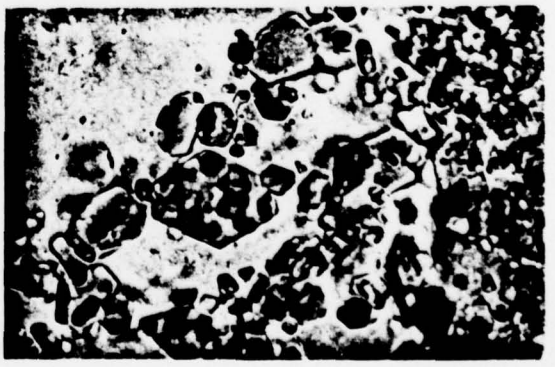


d.) Muridae

M. musculus
(House mouse)

e.) Muridae

M. musculus
(House mouse)



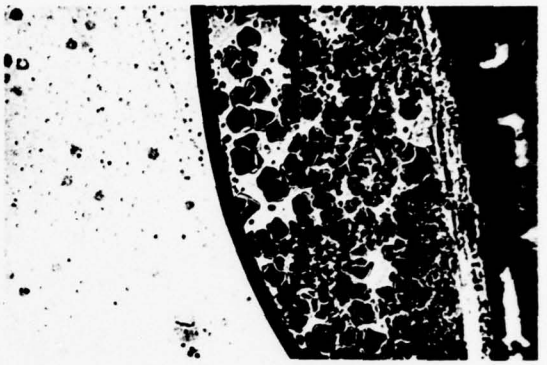
f.) Muridae

M. musculus
(House mouse)

App. 25: class: MAMMALIA; order: RODENTIA

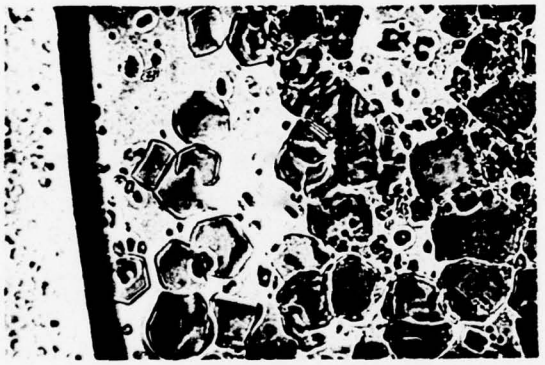
a.) Muridae

M. musculus
(House mouse)



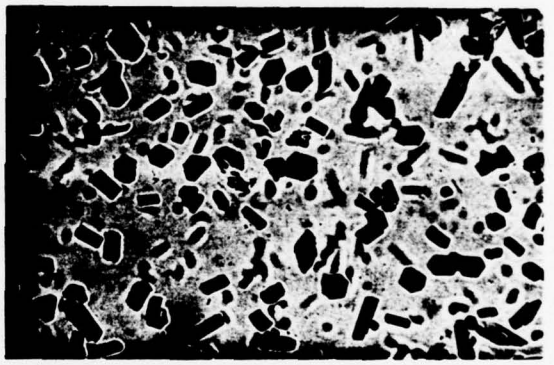
b.) Muridae

Rattus norvegicus
(Norway rat)



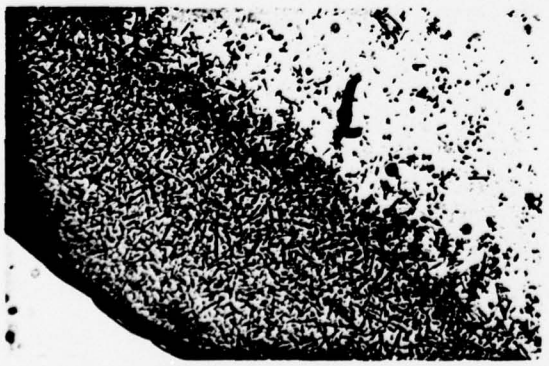
c.) Muridae

R. norvegicus
(Norway rat)



d.) Muridae

R. norvegicus
(Norway rate)



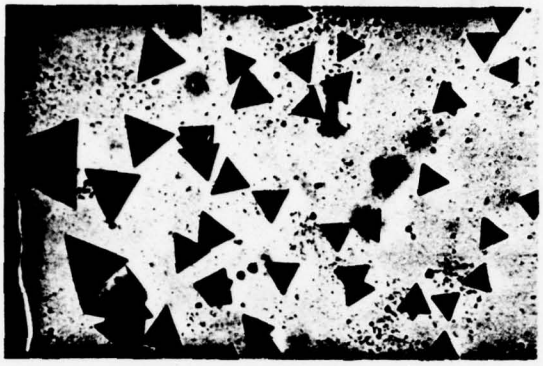
e.) Muridae

R. norvegicus
(Norway rat)

f.) Erethizontidae

Erethizon dorsatum
(Porcupine)

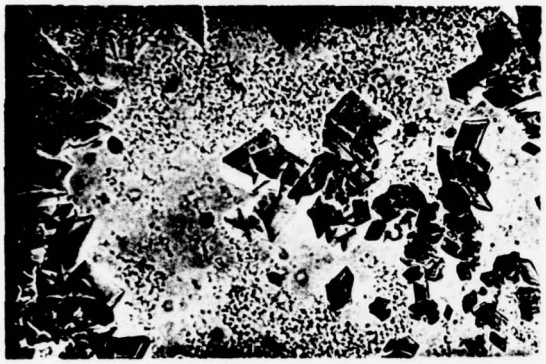
App. 26: class: MAMMALIA; order RODENTIA



a.) Cavidae

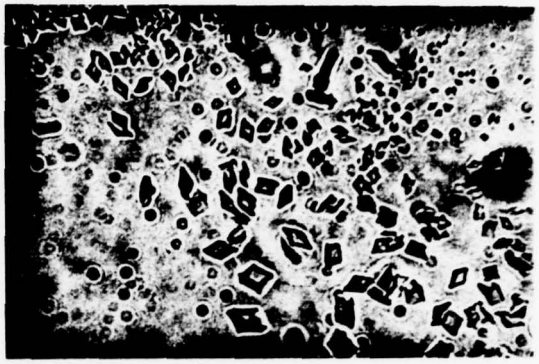
Cavia porcellus
(Guinea pig

App. 27: class: MAMMALIA; order: LAGOMORPHA



a.) Leporidae

Lepus californicus
(Black-tailed jack rabbit)



b.) Leporidae

Sylvilagus audubonii
(Desert cottontail)

c.) Leporidae

S. audubonii
(Desert cottontail

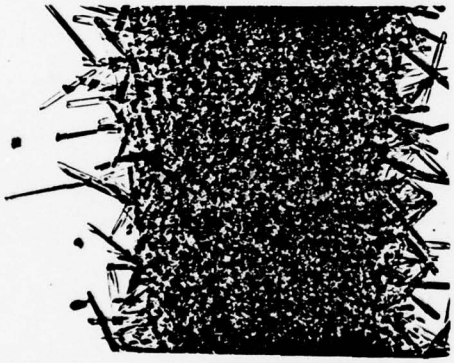
App. 28: class: MAMMALIA; order: CARNIVORA

a.) Canidae

Canis familiaris
(Dog)

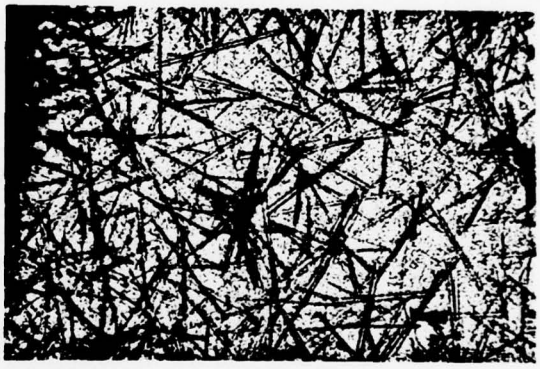
b.) Canidae

C. familiaris
(Dog)



c.) Canidae

C. latrans
(Coyote



d.) Canidae

C. lupus columbianus
(Wolf)

e.) Canidae

C. lupus columbianus
(Wolf)

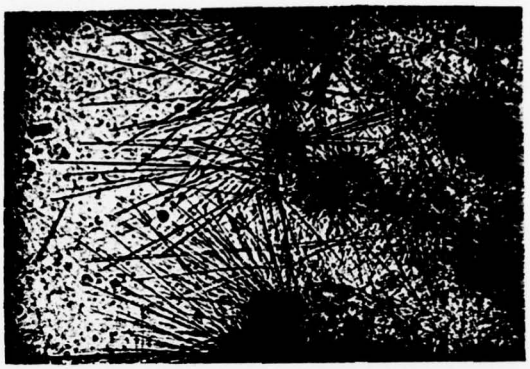
f.) Canidae

C. lupus columbianus
(Wolf)

App. 29: class: MAMMALIA; order: CARNIVORA

a.) Canidae

C. familiaris dingo
(Dingo)

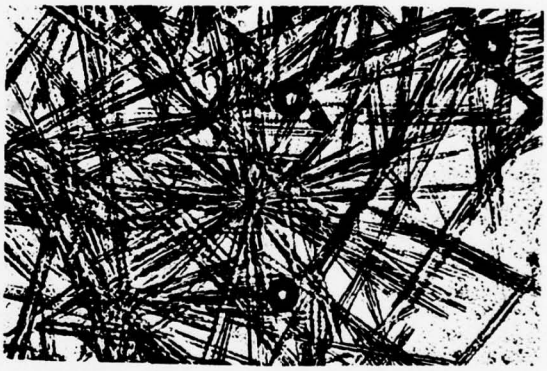


b.) Canidae

C. aureus syriacus
(Golden jackel)

c.) Canidae

C. aureus syriacus
(Golden jackel)



d.) Canidae

Urocyon cinereoargenteus californicus
(Grey fox)

e.) Canidae

U. cinereoargenteus californicus
(Grey fox)

f.) Canidae

Chrysocyon brachyurus
(Maned wolf)

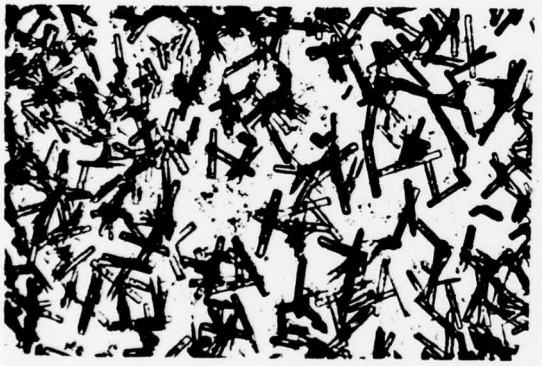
App. 30: class: MAMMALIA; order: CARNIVORA

a.) Canidae

C. brachyurus
(Maned wolf)

b.) Canidae

Speothos venaticus venaticus
(Bush dog)

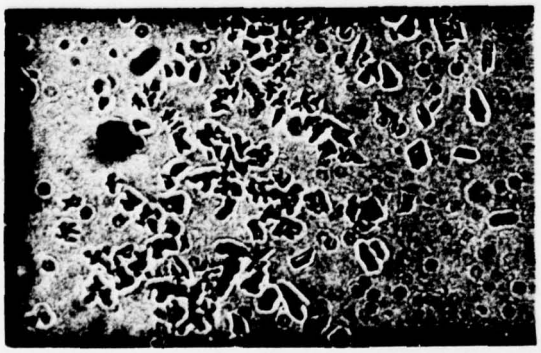


c.) Canidae

Fennecus zerda
(Fennec fox)

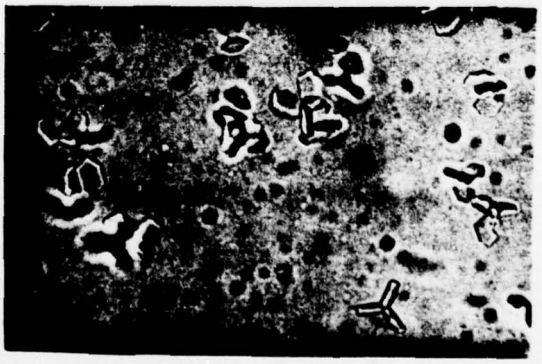
d.) Ursidae

Tremarctos ornatus
(Spectacled bear)



e.) Ursidae

Ursus americanus
(Black bear)



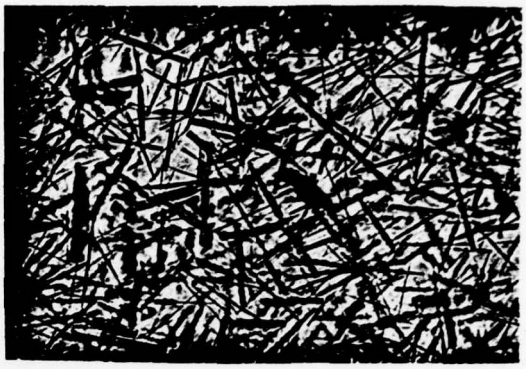
f.) Procyonidae

Procyon lotor
(Racoon)

App. 31: class: MAMMALIA; order: CARNIVORA

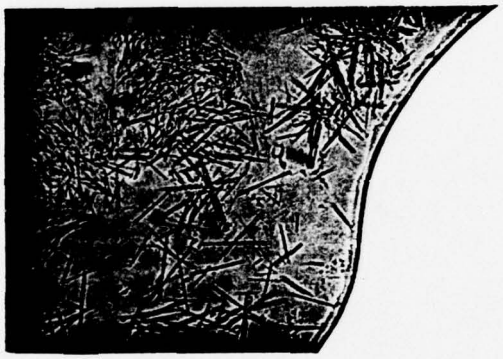
a.) Taxidea

Mephitis mephitis
(Striped skunk)



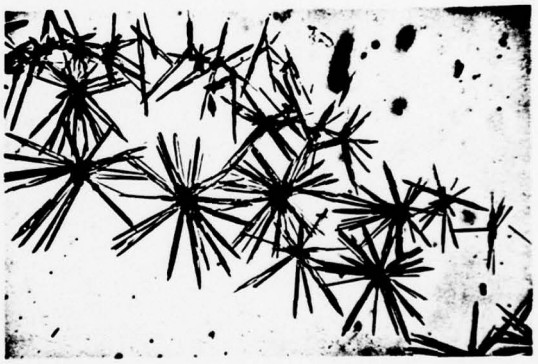
b.) Taxidea

M. mephitis
(striped skunk)



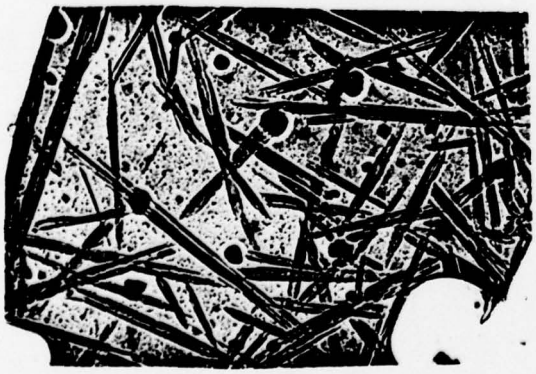
c.) Mustelidea

Mustela putorius
(European polecat)



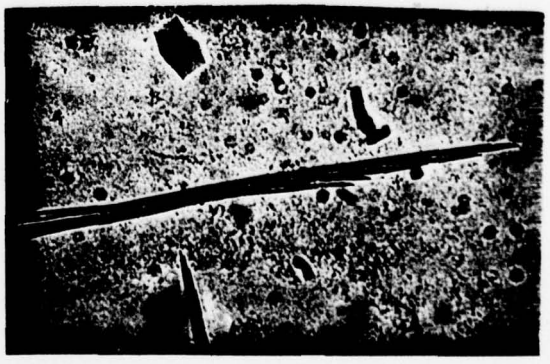
d.) Felidae

Felis catus
(Domestic cat)



e.) Felidae

F. catus
(Domestic cat)



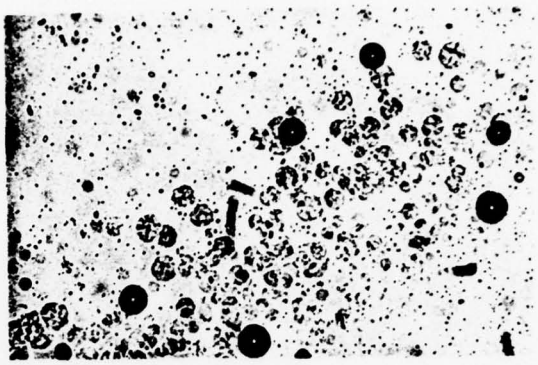
f.) Felidae

F. catus
(Domestic cat)

App. 32: class: MAMMALIA; order: CARNIVORA

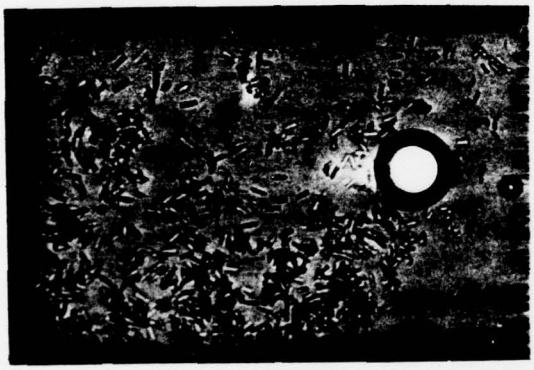
a.) Felidea

F. concolor
(Puma)



b.) Felidea

F. silvestris silvestris
(Russian wild cat)



c.) Felidea

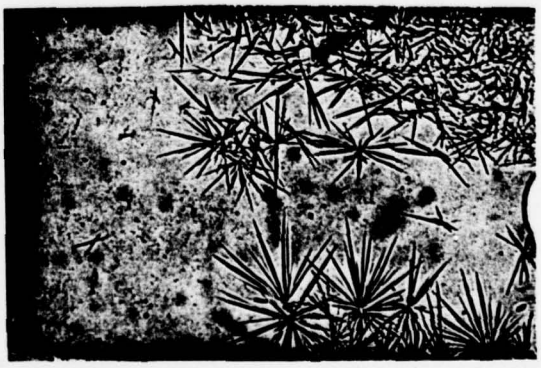
F. serval ingridi
(Serval cat)

d.) Felidea

F. serval ingridi
(serval cat)

e.) Felidea

Panthera leo
(Lion)



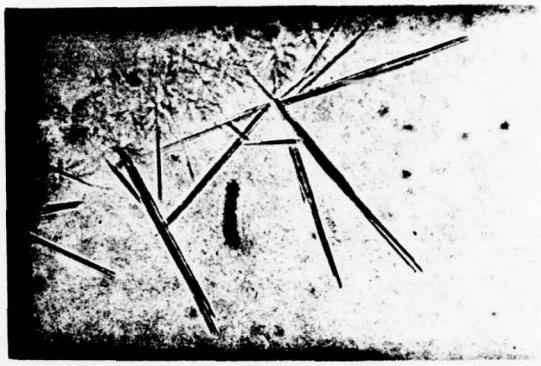
f.) Felidea

P. tigris tigris
(Bengal tiger)

App: 33: class: MAMMALIA; order: CARNIVORA

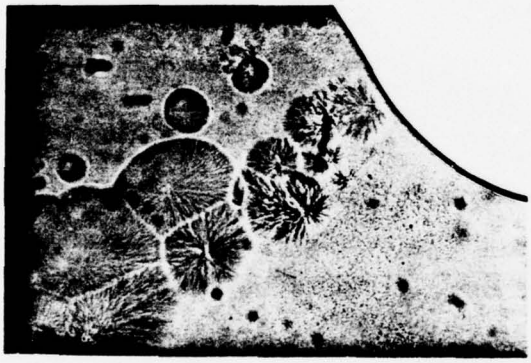
a.) Felidea

P. tigris altaica
(Siberian tiger)



b.) Felidea

P. tigris altaica
(Siberian tiger)

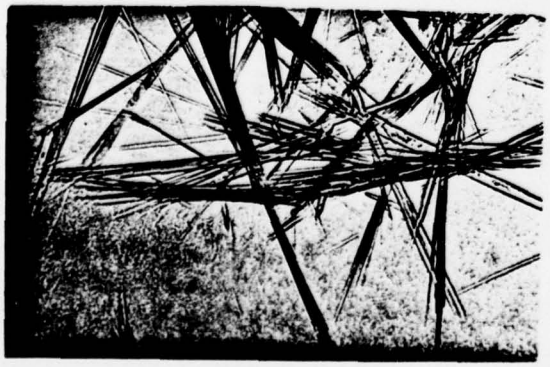


c.) Felidea

P. onca
(Jaguar)

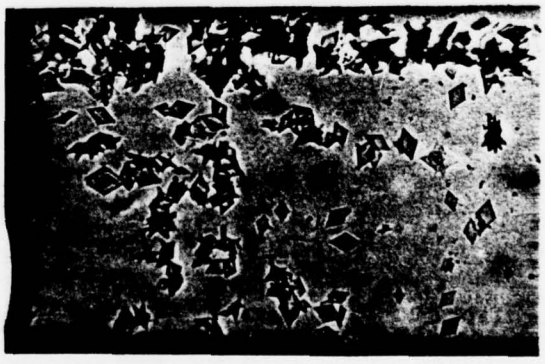
d.) Felidea

P. onca
(Juguar)



e.) Felidea

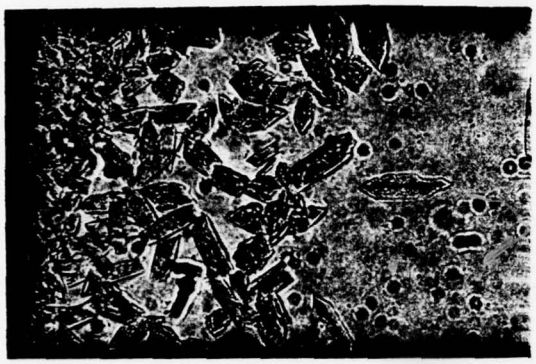
P. pardus delacouri
(Leopard)



f.) Felidea

P. pardus japonensis
(Leopard)

App. 34: class: MAMMALIA; order: CARNIVORA

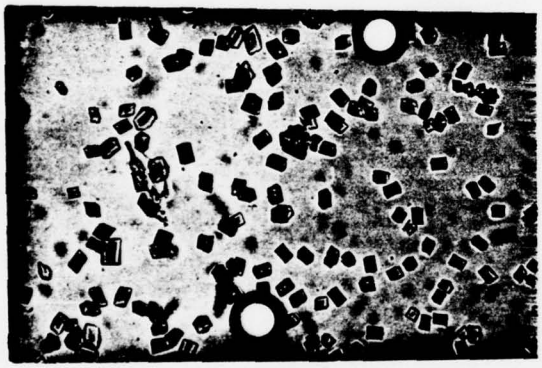


a.) Felidea

Acinonyx jubatus
(Cheetah)

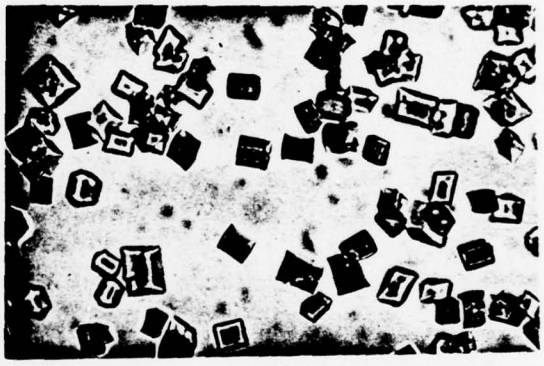
b.) Felidea

A. jubatus
(Cheetah)



c.) Felidea

Panthera (neofelis) nebulosa
(Clouded leopard)



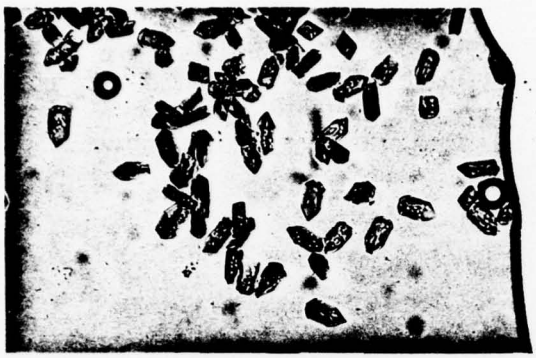
d.) Felidea

P. (neofelis) nebulosa
(Clouded leopard)

e.) Felidea

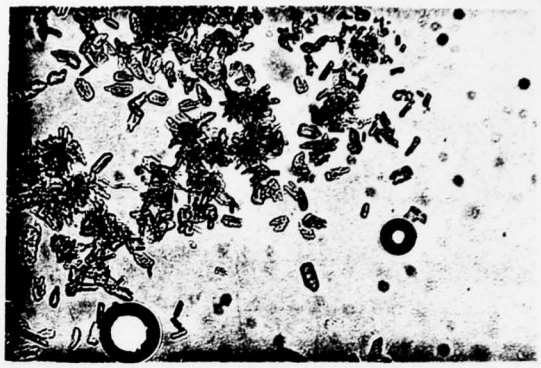
P. (neofelis) nebulosa
(Clouded leopard)

App. 35: class: MAMMALIA; order: PINNIPEDIA



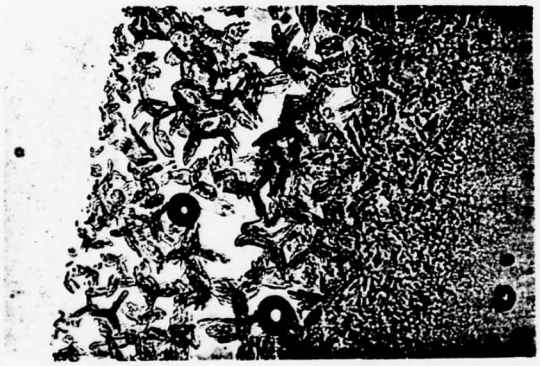
a.) Otariidae

Arctocephalus australis
(Fur seal)



b.) Otariidae

Zalophus claifornicus
(California sea lion)



b.) Otariidae

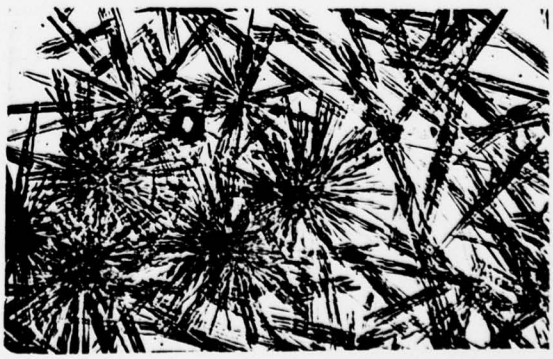
Z. californicus
(California sea lion)

d.) Otariidae

Z. californicus
(California sea lion)

e.) Otariidae

Callorhinus ursinus
(Northern fur seal)



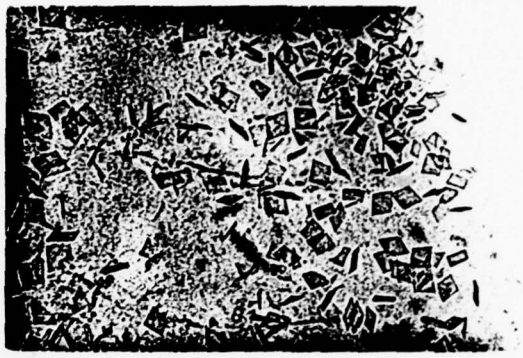
f.) Otariidae

C. ursinus
(Northern fur seal)

App. 36: class: MAMMALIA; order: PINNIPEDIA

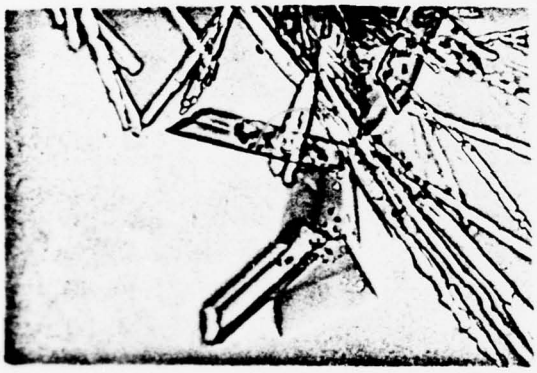
a.) Otariidae

C. ursinus
(Northern fur seal)



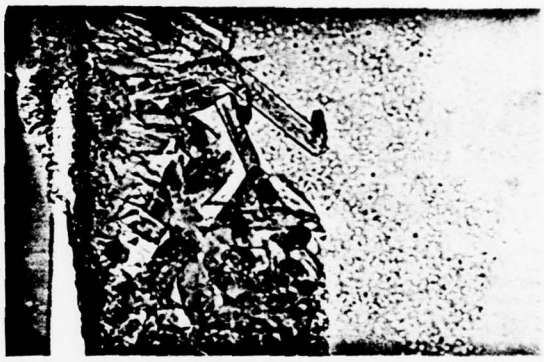
b.) Phocidae

Halichoerus grypus
(Gray seal)



c.) Phocidae

Mirounga angustirostris
(Northern elephant seal)



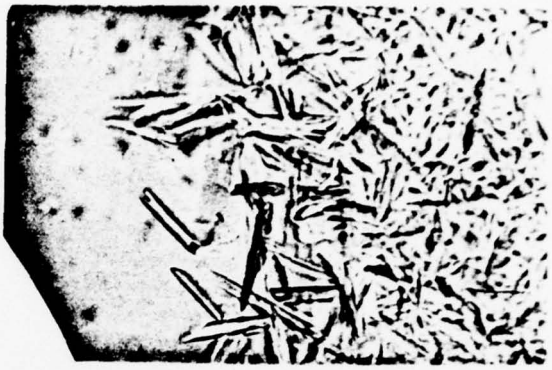
d.) Phocidae

Mirounga angustirostris
(Northern elephant seal)

App. 37: class: MAMMALIA; order: CETACEA

a.) Delphinidae

Tursiops gilli
(Gills bottle-nosed dolphin)



b.) Delphinidae

T. gilli
(Gills bottle-nosed dolphin)

c.) Delphinidae

T. gilli
(Gills bottle-nosed dolphin)

d.) Delphinidae

T. gilli
(Gills bottle-nosed dolphin

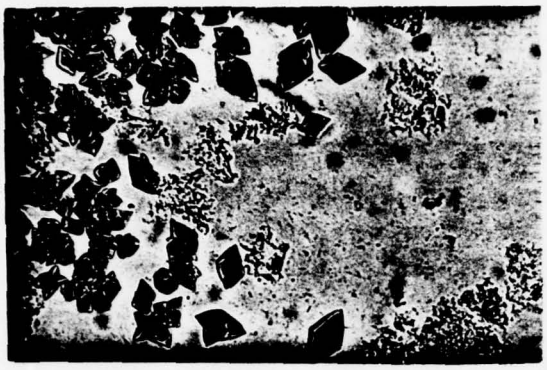
e.) Delphinidae

T. gilli
(Gills bottle-nosed dolphin)

f.) Delphinidae

T. gilli
(Gills bottle-nosed dolphin)

App. 38: class: MAMMALIA; order: CETACEA



a.) Delphinidae

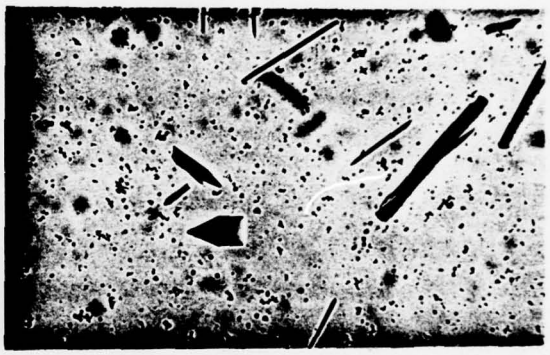
T. gilli
(Gills Bottle-nosed dolphin)

b.) Delphinidae

Tursiops truncatus
(Bottle-nosed dolphin)

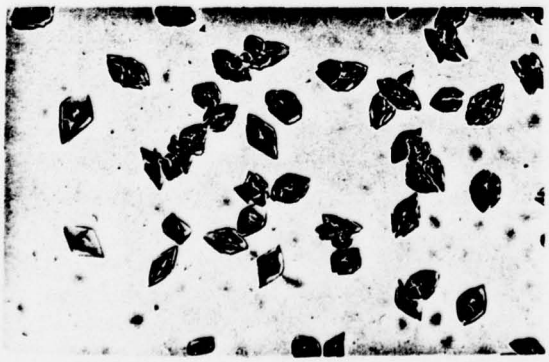
c.) Delphinidae

T. truncatus
(Bottle-nosed dolphin)



d.) Delphinidae

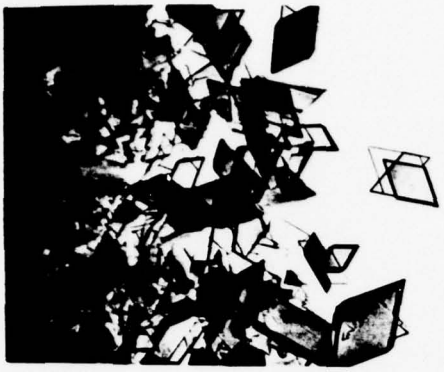
T. truncatus
(Bottle-nosed dolphin)



e.) Delphinidae

T. truncatus
(Bottle-nosed dolphin)

App. 39: class: MAMMALIA; order: PERISSODACTYLA



a.) Equidae

Equus caballus (horse)

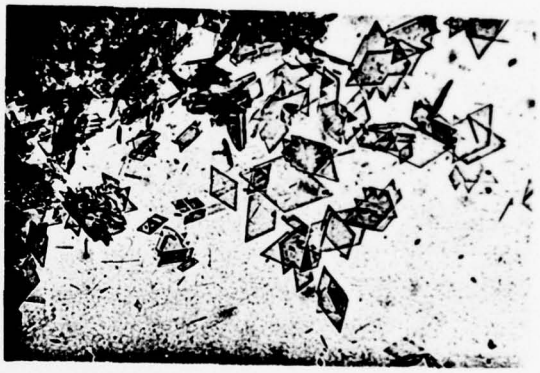
b.) Equidae

Equus prezewalskii

(Prezewalskis wild horse)

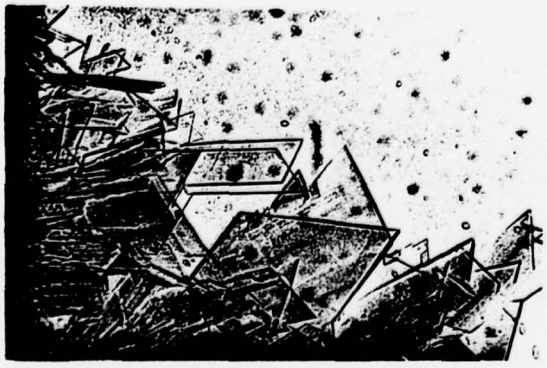
c.) Equidae

E. prezewalskii
(Prezewalskis wild horse)



d.) Equidae

E. zebra hartmani
(Hartmans mt. zebra)



e.) Equidae

E. zebra hartmani
(Hartmans mt. zebra)

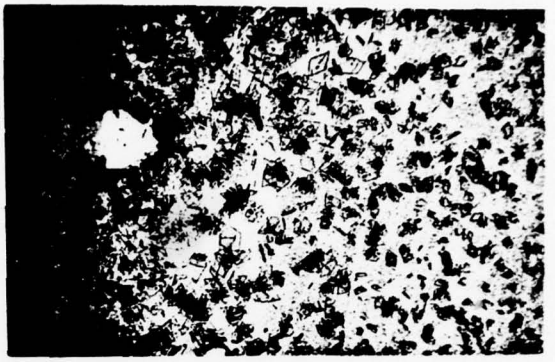
f.) Equidae

E. asinus asinus
(Domestic ass)

App. 40: class: MAMMALIA; order: PERISSODACTYLA

a.) Equidae

Equus asinus africanus

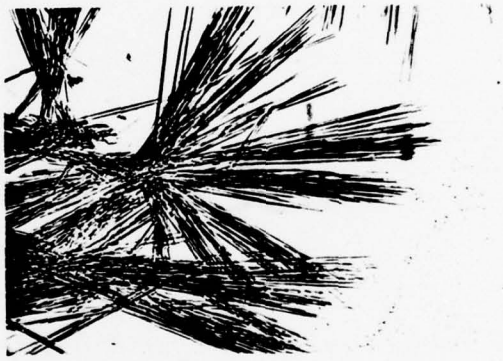


b.) Tapiridae

Tapirus bairdi
(Bairds tapir)

c.) Rhinocerotidae

Rhinoceros unicornis
(Great Indian rhinoceros)



d.) Rhinocerotidae

R. unicornis
(Great Indian rhinoceros)

e.) Rhinocerotidae

R. unicornis
(Great Indian rhinoceros)

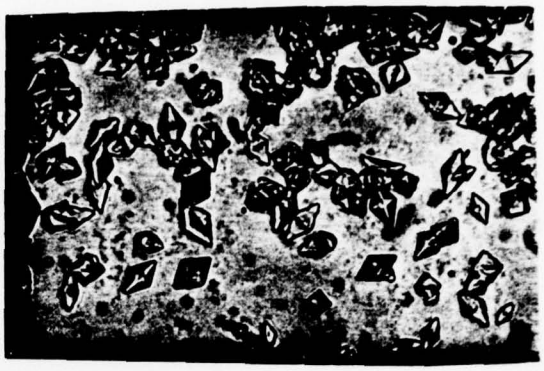
f.) Rhinocerotidae

Ceratotherium simum simum
(S. white Rhinoceros)

App. 41: class: MAMMALIA; order: HYRACOIDEA

a.) Procaviidae

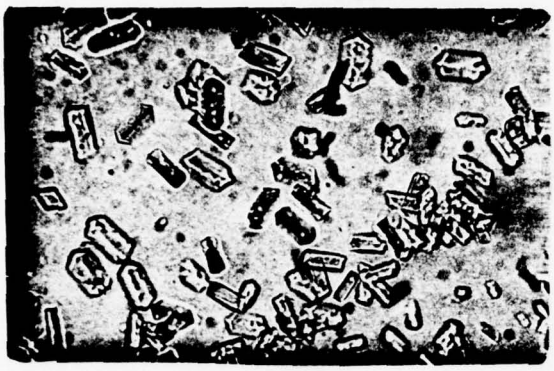
Procavia capensis capensis
(South African dassie)



b.) Procaviidae

Procavia capensis capensis
(South African dassie)

App. 42: class: MAMMALIA; order: ARTIODACTYLA

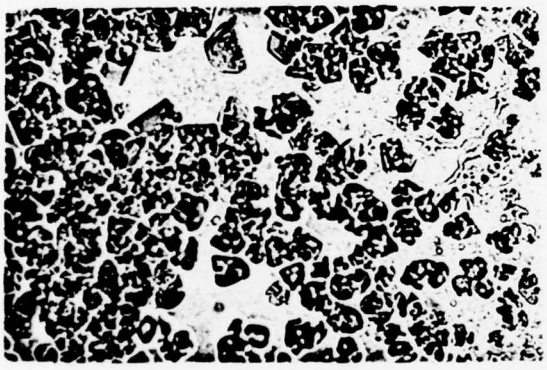


a.) Suidae

Sus scrofa (pig)

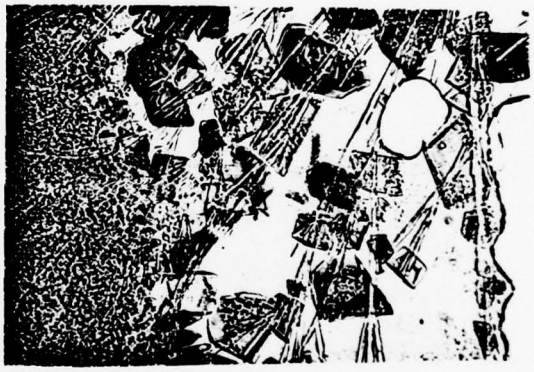
b.) Tayassuidae

Tayassu tajacu sonoriensis (colared peccary)



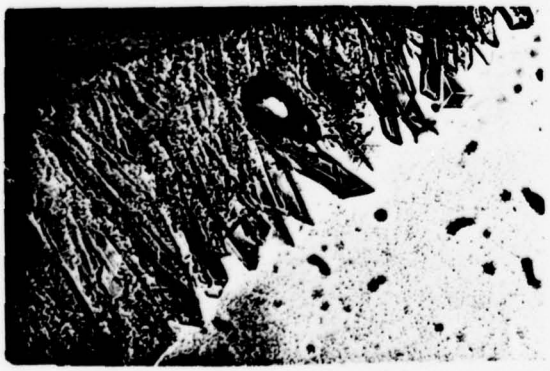
c.) Tayassuidae

T. tajacu sonoriensis (colared peccary)



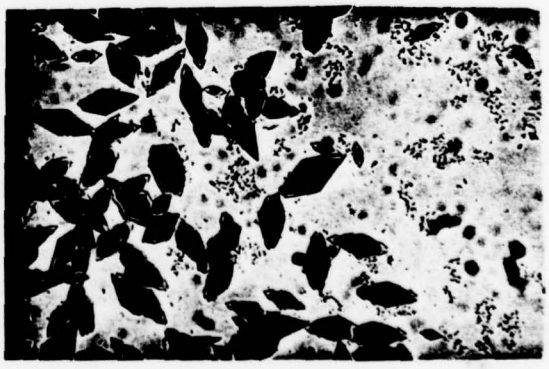
d.) Tayassuidae

T. tajacu sonoriensis (colared peccary)



e.) Hippopotamidae

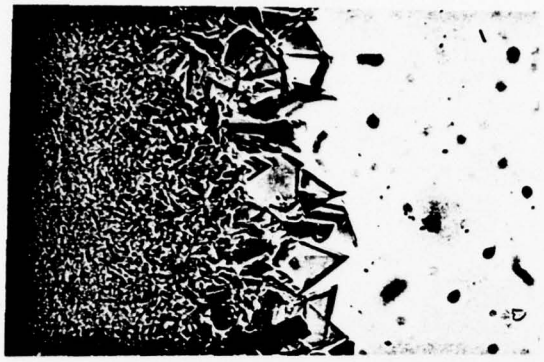
Choeropsis liberiensis (pigmy hippopotamus)



f.) Hippopotamidae

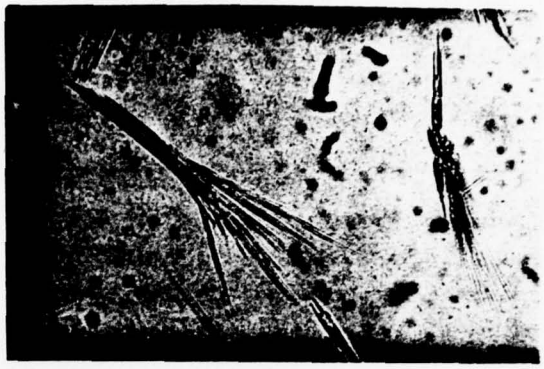
C. liberiensis (pigmy hippopotamus)

App.43: class: MAMMALIA; order: ARTIODACTYLA



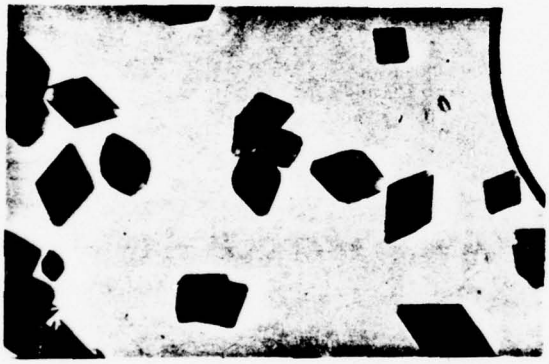
a.) Hippopotamidae

C. liberiensis (pigmy hippopotamus)



b.) Camelidae

Camelus dromedarius (Camel)

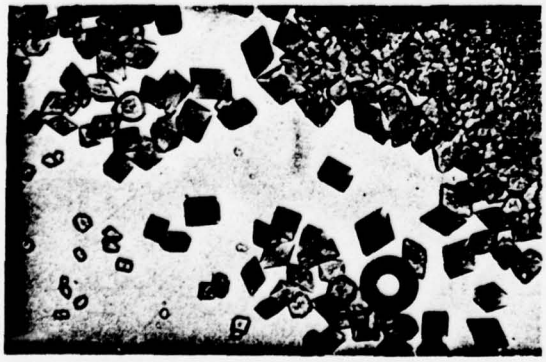


c.) Camelidae

Lama glama guanicoe (Guanaco)

d.) Camelidae

L. glama guanicoe (Guanaco)



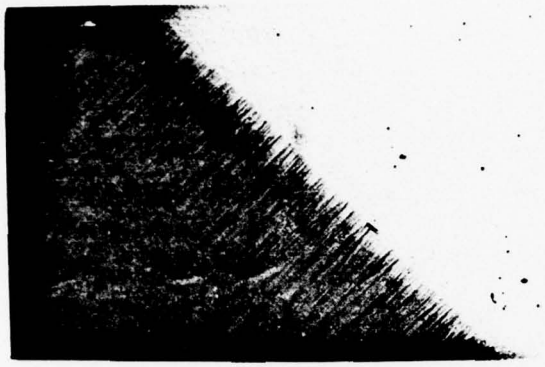
e.) Camelidae

L. glama glama (Llama)

f.) Camelidae

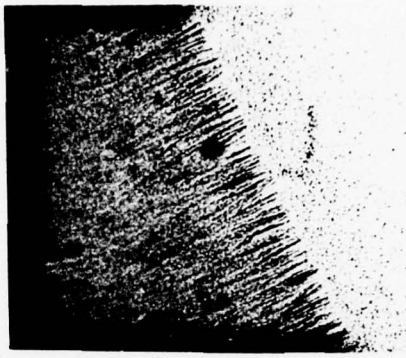
L. glama glama (Llama)

App. 44 : class; MAMMALIA; order: ARTIODACTYLA



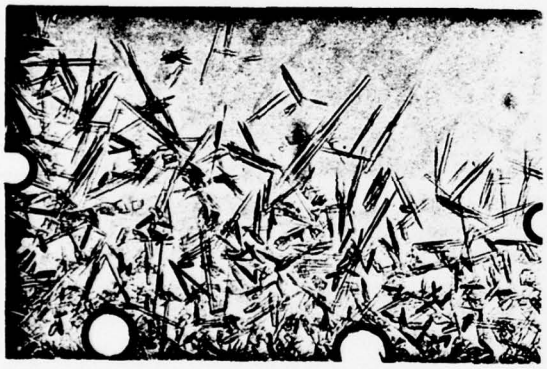
a.) Cervidae

Muntiacus reevesi reevesi (Reeves) muntjac)



b.) Cervidae

Mazana americana
(Red Brocket deer)



c.) Cervidae

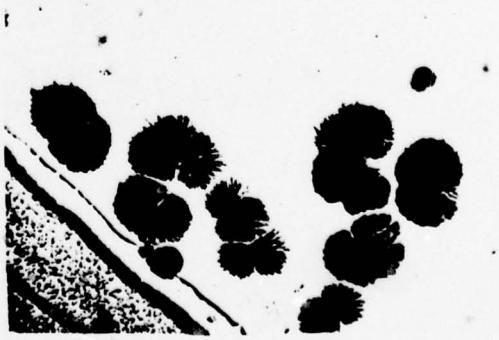
Cervus elaphus songaricus
(Altai wapti)

d.) Cervidae

Cervus nipon pseudaxis
(Sika deer)

e.) Cervidae

Odocoileus hemionus columbiana
(Black-tailed deer)



f.) Cervidae

Odocoileus heminous fuliginata
(Mule deer)

App. 45 : class: MAMMALIA; order: ARTIODACTYLA

a.) Cervidae

Rangifer taranadus
(N. European Reindeer)

b.) Cervidae

R. taranadus (Reindeer)

c.) Antilocapriadae

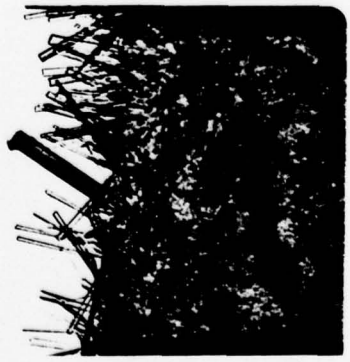
Antilocapra americana (Pronghorn)

d.) Antilocapriadae

A. americana (Pronghorn)

e.) Antilocapriadae

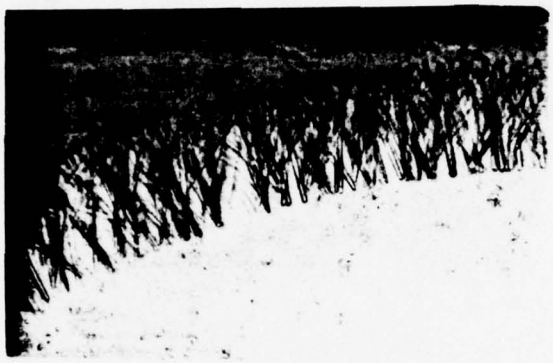
A. americana (Pronghorn)



f.) Bovidae

Oryx leucoryx (Arabian oryx)

App. 46: class: MAMMALIA; order: ARTIODACTYLA



a.) Bovidae

Oryx dammah (Scimitar horned oryx)

b.) Bovidae

O. dammah (Scimitar horned oryx)

c.) Bovidae

O. dammah (scimitar horned oryx)

d.) Bovidae

O. dammah (Scimitar horned oryx)

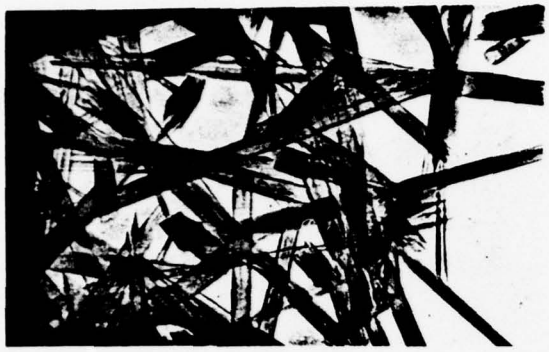
e.) Bovidae

O. dammah (Scimitar horned oryx)

f.) Bovidae

O. dammah (Scimitar horned oryx)

App. 47: class: MAMMALIA; order:ARTIODACTYLA



a.) Bovidae

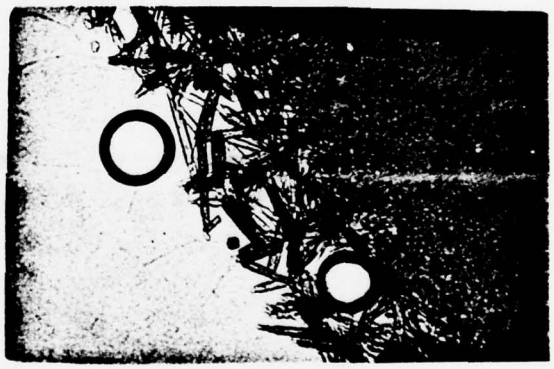
Oryx dammah (Scimitar horned oryx)

b.) Bovidae

Connochaetes

c.) Bovidae

C. taurinus albojubatus
(E. Whitebeard wildebeest)



d.) Bovidae

C. taurinus albojubatus
(E. Whitebread wildebeest)

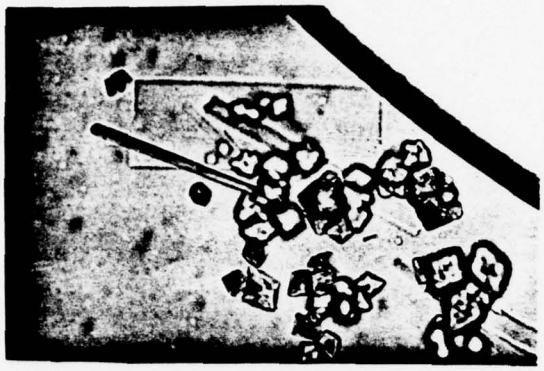
e.) Bovidae

C. taurinus albojubatus
(E. Whitebeard wildebeest)

f.) Bovidae

C. taurinus albojubatus
(E. Whitebeard wildebeest)

App. 48 : class: MAMMALIA; order: ARTIODACTYLA

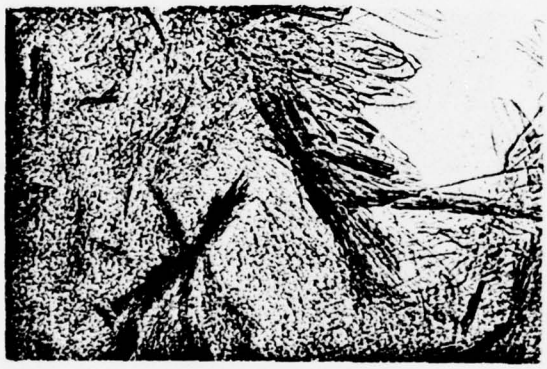


a.) Bovidae

Connochaetes taurinus albojubatus
(E. Whitebeard wildebeest)

b.) Bovidae

C. taurinus albojubatus
(E. Whitebeard wildebeest)



c.) Bovidae

C. taurinus albojubatus

d.) Bovidae

Addax nasomeculatus (Addax)

e.) Bovidae

Addax nasomaculatus (Addax)

f.) Bovidae

Damaliscus dorcus dorcus
(Bontebok)

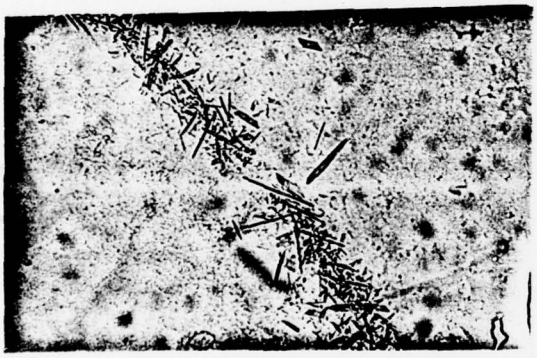
App. 49 : class; MAMMALIA; order: ARTIODACTYLA

a.) Bovidae

Damaliscus dorcus dorcus
(Bontebok)

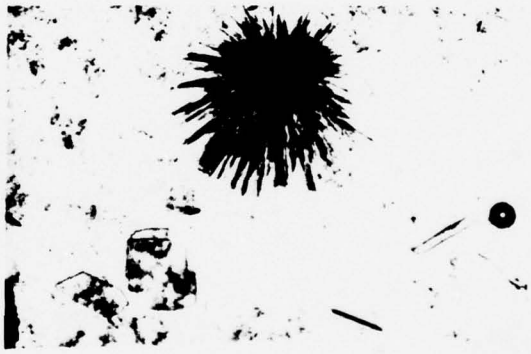
b.) Bovidae

D. dorucs dorcus (Bontebok)



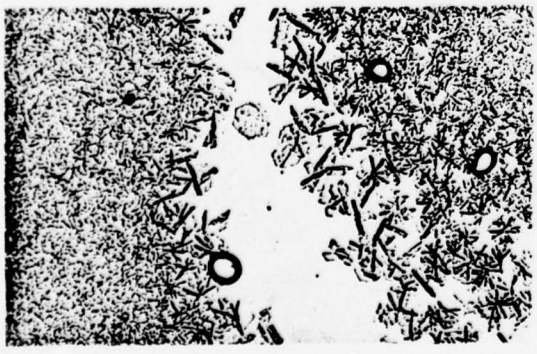
c.) Bovidae

D. dorcus
(Bontebok + Blesbok)



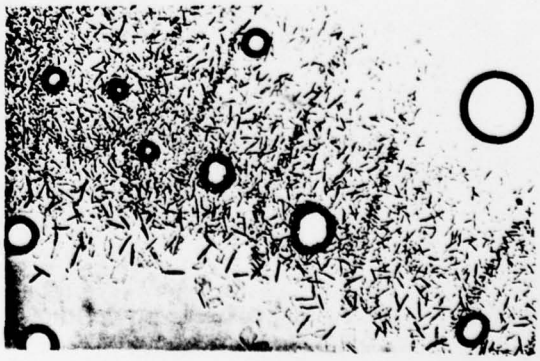
d.) Bovidae

D. dorcus
(Bontebok + Blesbok)



e.) Bovidae

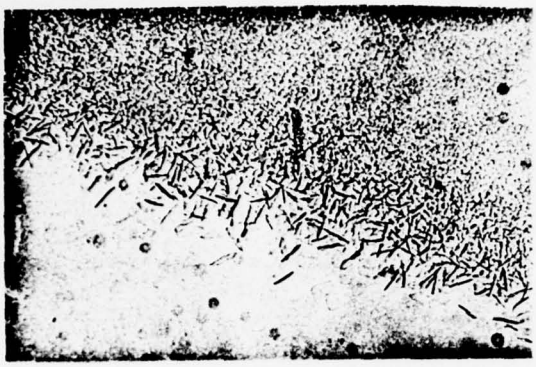
D. dorcus
(Bontebok + Blesbok)



f.) Bovidae

D. dorcus
(Bontebok + Blesbok)

App.50 : class: MAMMALIA; order: ARTIODACTYLA

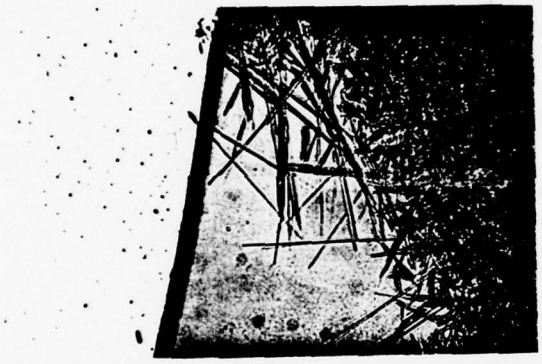


a.) *Bovidae*

Hippotragus niger (Sable antelope)

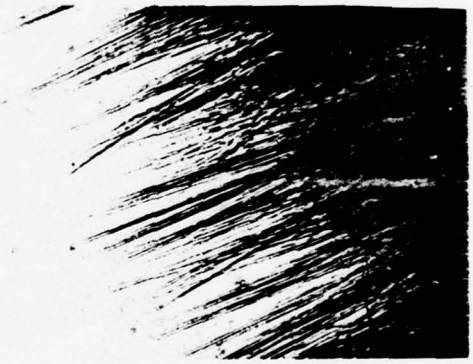
b.) Bovidae

Hippotragus equinus cottoni
(Roan antelope)



c.) Bovidae

Antilope cervicapra (Blackbuck)



d.) Bovidae

Antidorcas marsupialis marsupialis
(S. African springbok)

e.) Bovidae

Gazella dama (Dama gazelle)

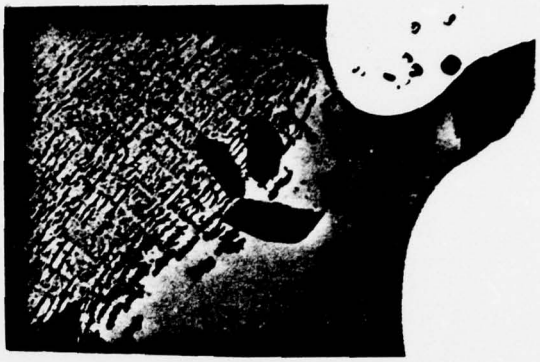
f.) Bovidae

Gazella granti granti
(Grants gazelle)

App. 51: class: MAMMALIA; order: ARTIODACTYLA

a.) Bovidae

Gazella granti roosevelti
(Grants gazelle)

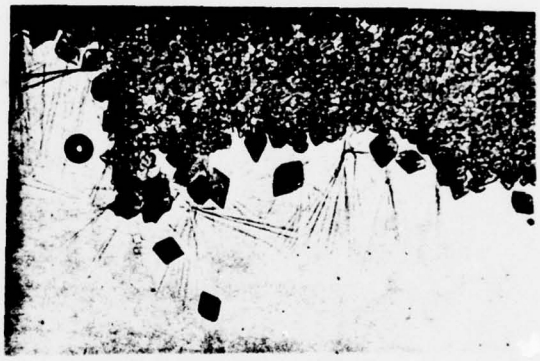


b.) Bovidae

G. granti roosevelti (Grants gazelle)

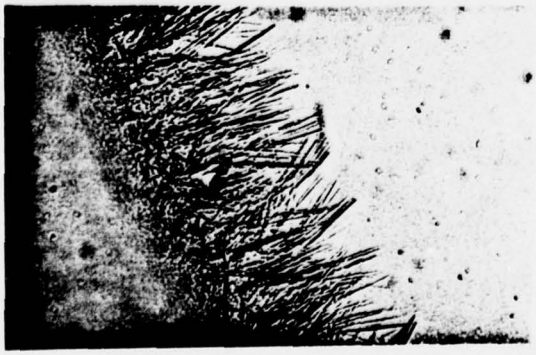
c.) Bovidae

G. granti roosevelti (Grants gazelle



d.) Bovidae

Aedyceros melampus rendilis
(Impala)



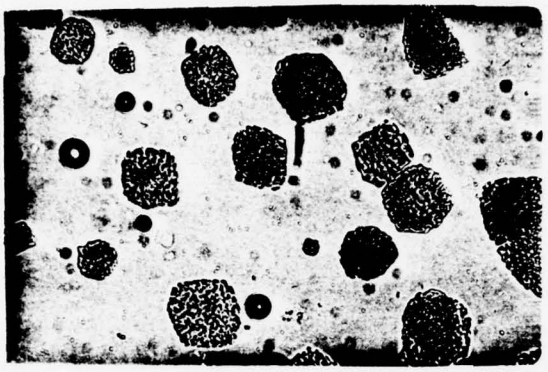
e.) Bovidae

Cephalophus sylvicultor sylvicultor
(Yellow-backed Duiker)

f.) Bovidae

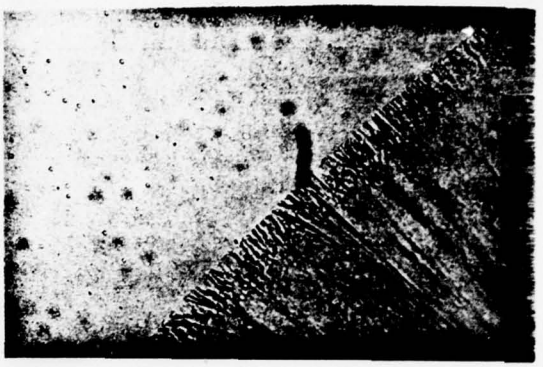
E. sylvicultor sylvicultor
(Yellow-backed Duiker)

App. 52 : class: MAMMALIA; order: ARTIODACTYLA



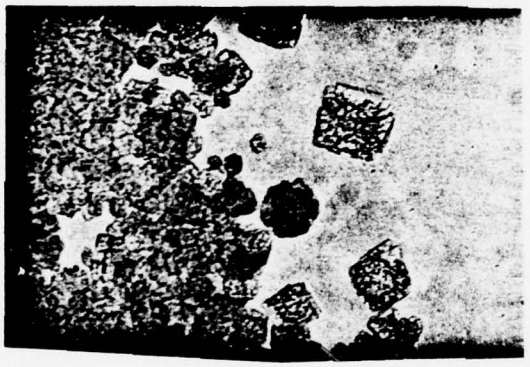
a.) Bovidae

Taurotragus (Eland)



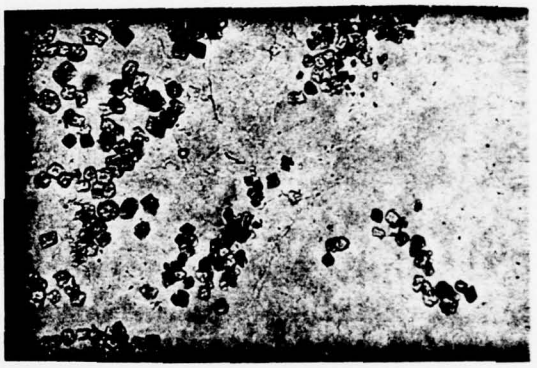
b.) Bovidae

Boselaphus tragocamelus (Nilgai)



c.) Bovidae

Tragelaphus spekei spekei
(E. African sitatunga)



d.) Bovidae

T. spekei spekei
(E. African sitatunga)

e.) Bovidae

Bison bison bison
(Prairie bison)

f.) Bovidae

Bison bonasus bonasus
(Wisent)

App. 53: class: MAMMALIA; order: ARTIODACTYLA

a.) Bovidae

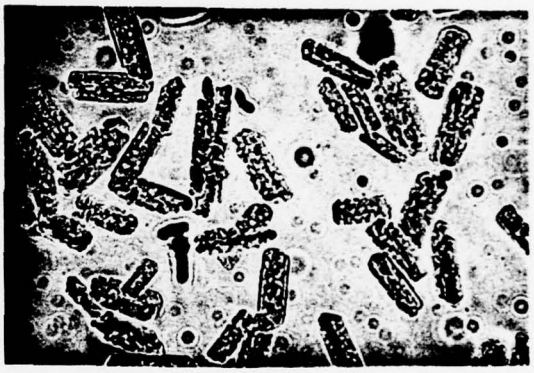
Bison bonasus bonasus
(Wisent)

b.) Bovidae

B. bonasus bonasus
(Wisent)

c.) Bovidae

Bison bonasus bonasus
(Wisent)

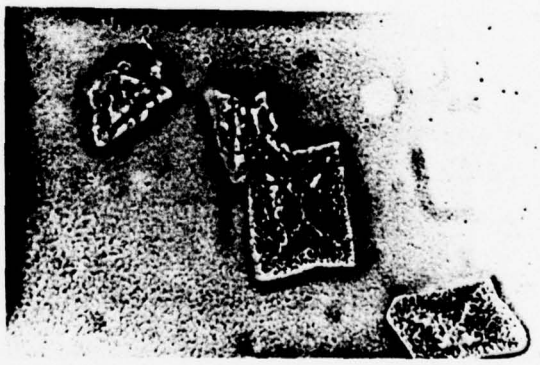


d.) Bovidae

Bos taurus (Domestic cow)

e.) Bovidae

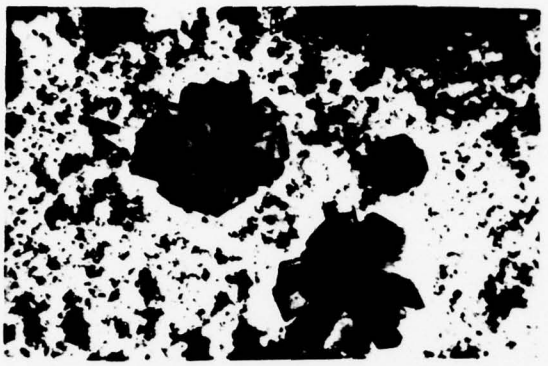
B. taurus (Domestic cow-Jersey)



f.) Bovidae

B. taurus (Domestic cow-Brangus)

App. 54 : class: MAMMALIA; order: ARTIODACTYLA



a.) Bovidae

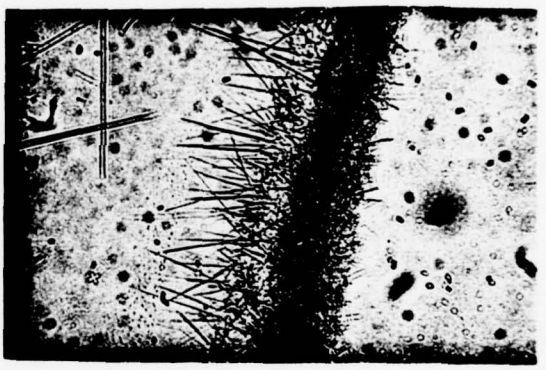
Bos taurus (Domestic cow)

b.) Bovidae

Bison bison + Bos taurus (Beefalo)

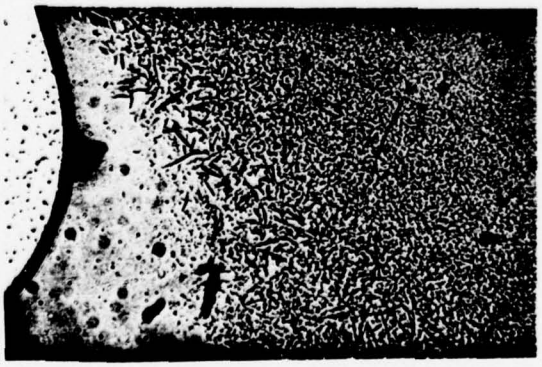
c.) Bovidae

Ovis aries (Domestic sheep-Suffolk)



d.) Bovidae

O. aries (Domestic sheep)



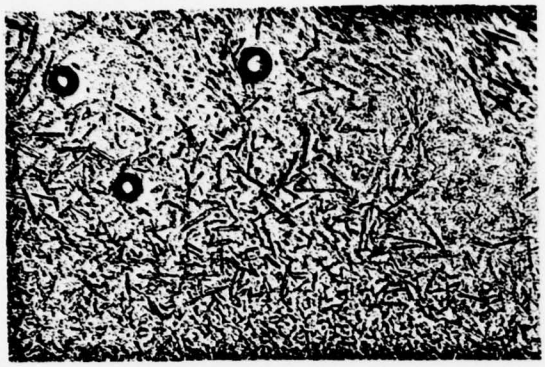
e.) Bovidae

O. aries (Domestic sheep)

f.) Bovidae

O. aries (Domestic sheep)

App. 55: Class: MAMMALIA; order: ARTIODACTYLA



a.) Bovidae

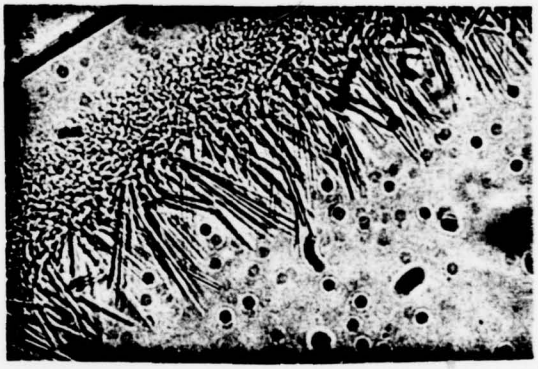
Ovis aries (Domestic sheep)

b.) Bovidae

O. aries (Domestic sheep)

c.) Bovidae

O. musimon (Mouflon)



d.) Bovidae

Capra aegagrus (Goat

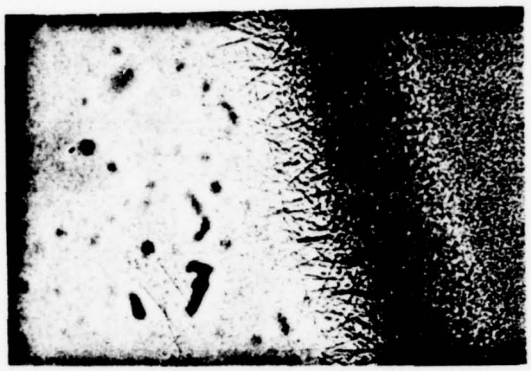
e.) Bovidae

C. aegagrus

f.) Bovidae

Capra aegagrus

App. 56: Class: MAMMALIA; order: ARTIODACTYLA

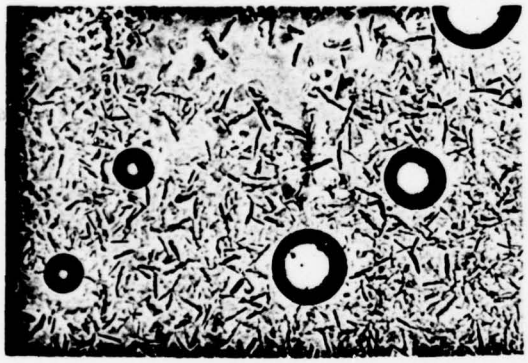


a.) Bovidae

C. aegagrus

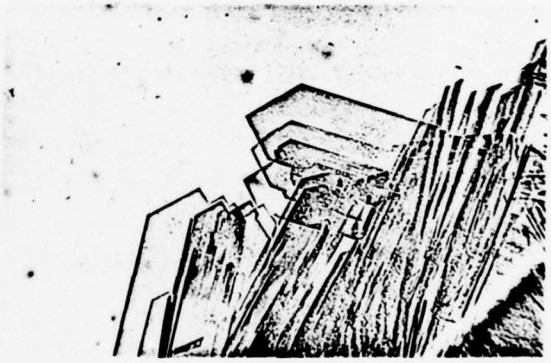
b.) Bovidae

C. falconeri heptneri
(Markhor)



c.) Bovidae

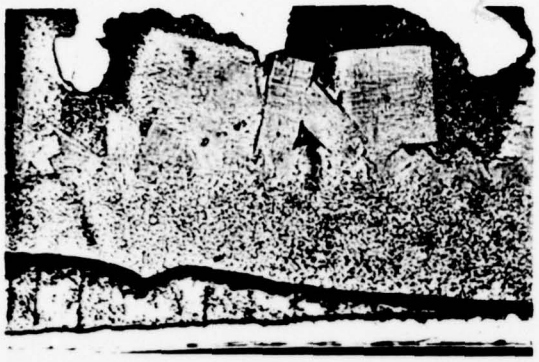
C. ibex severtzovi (Ibex)



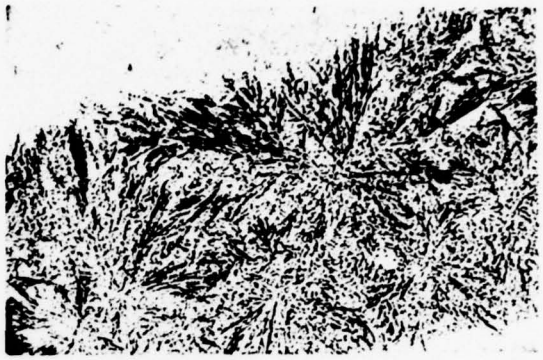
d.) Bovidae

Hemitragus jemlahicus
(Himabyan tahr)

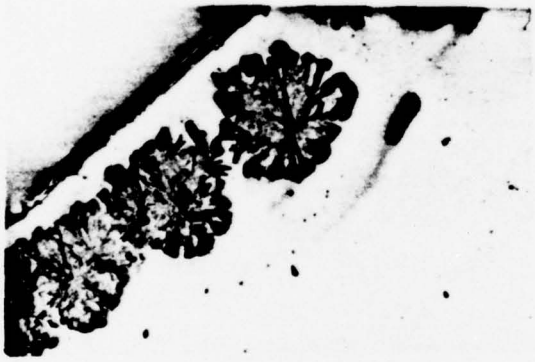
App. 57: class: AVES; order: RHEIFORMES, FALCONIFORMES



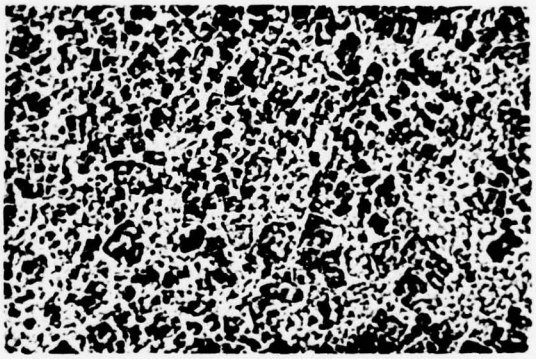
a.) Rheidae
Pterocnemia pennata pennata
(Darwin's Rhea)



b.) Rheidae
P. pennata pennata
(Darwin's Rhea)

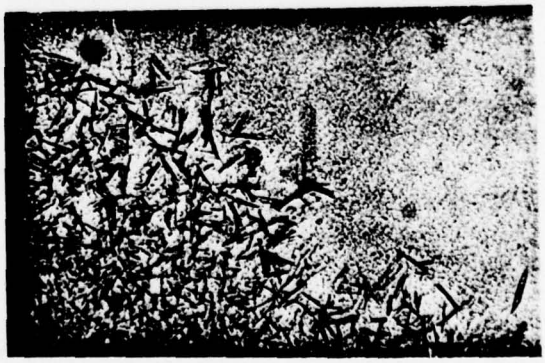


c.) Cathartidae
Cathartes aura septentrionalis



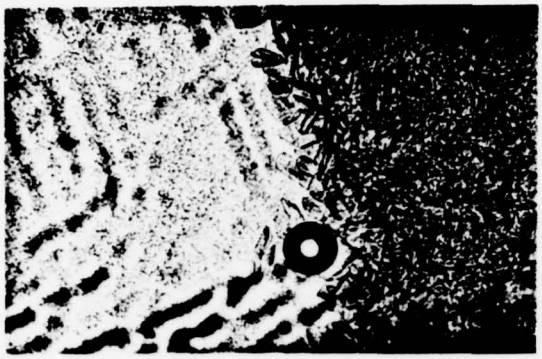
d.) Cathartidae
C. aura septentrionalis

e.) Cathartidae
C. aura septentrionalis
(E. turkey vulture



f.) Accipitridae
Elanus leucurus
(White tailed kite)

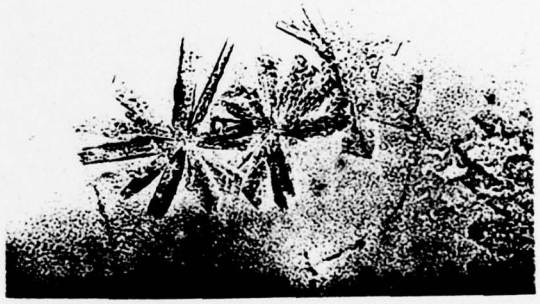
App. 58: class: AVES; order: FALCONIFORMES, ANSERIFORMES



a.) Accipitridae
Circus cyaneus
(Marsh hawk)

b.) Accipitridae
Morphnus guianensis
(Crested eagle)

c.) Falconidae
Falco sparverius
(Sparrow hawk)

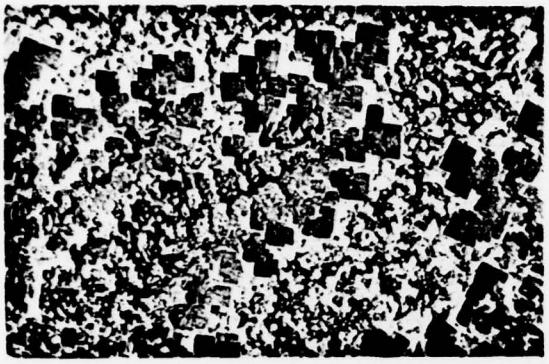


d.) Anatidae
Anser fabalis brachyrhynchus
(Pink-footed goose)

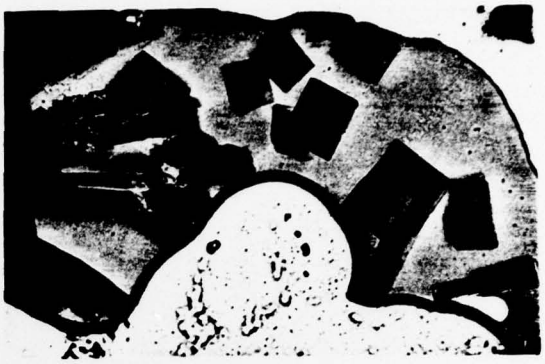
e.) Anatidae
A. fabalis brachyrhynchus
(Pink-footed goose)

f.) Anatidae
A. caerulescens
(Snow goose)

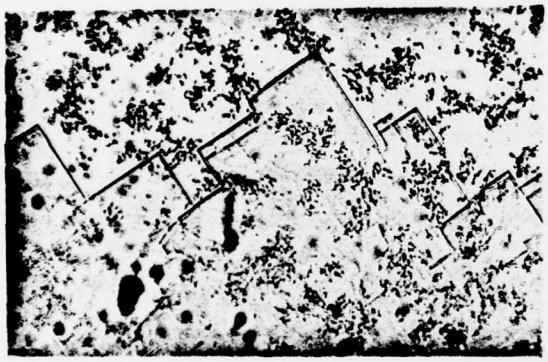
App. 59: class: AVES; order: ANSERIFORMES, GALLIFORMES



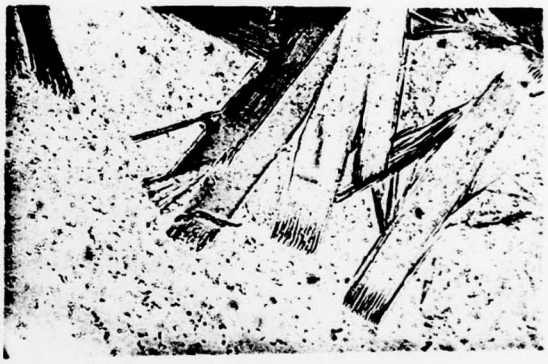
a.) Anatidae
Anser caerulescens
(Snow goose)



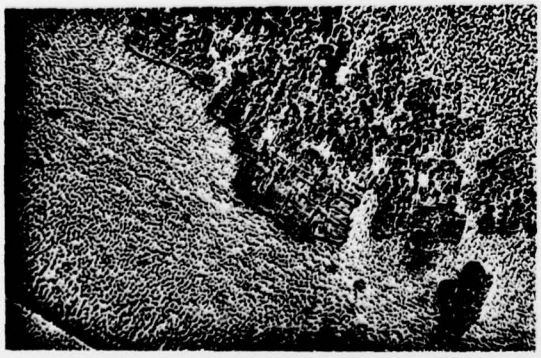
b.) Anatidae
A. caerulescens
(Snow goose)



c.) Anatidae
Branta leucopsis
(Barnacle goose)



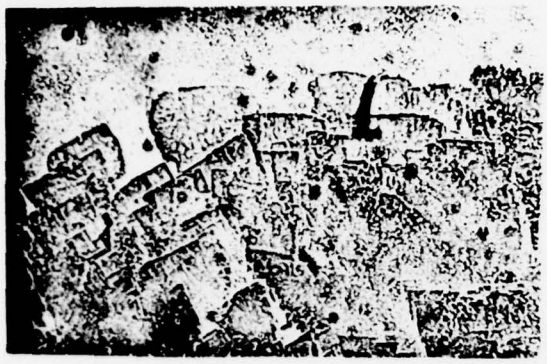
d.) Anatidae
B. leucopsis
(Barnacle goose)



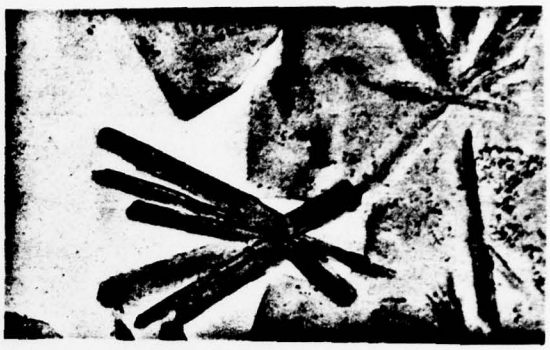
e.) Phasianidae
Phasianus colchicus
(Ring-neck pheasant)

f.) Phasianidae
P. colchicus
(Ring-neck pheasant)

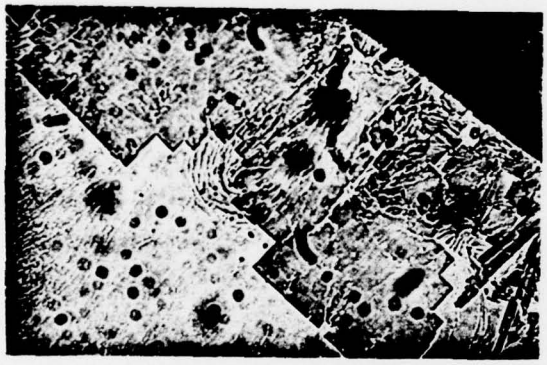
App. 60: class: AVES; order: GALLIFORMES



a.) Phasianidae
Chrysolophus pictus
(Golden pheasant)

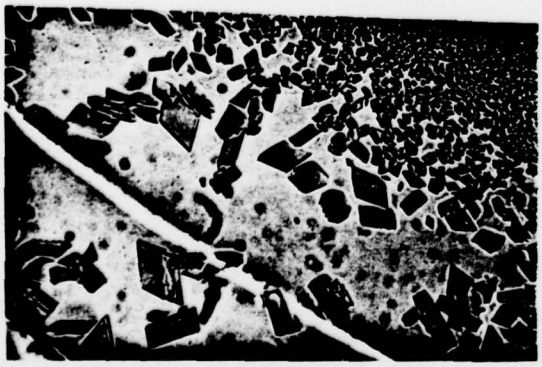


b.) Phasianidae
Lophura swainhoii
(Swinhoe's pheasant)

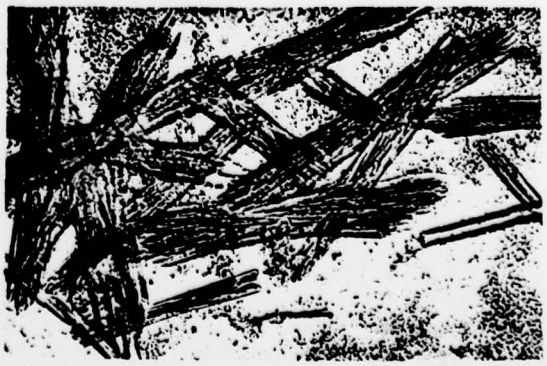


c.) Phasianidae
Colinus virginianus
(Bob white)

d.) Phasianidae
Meleagris galopayo
(Turkey)

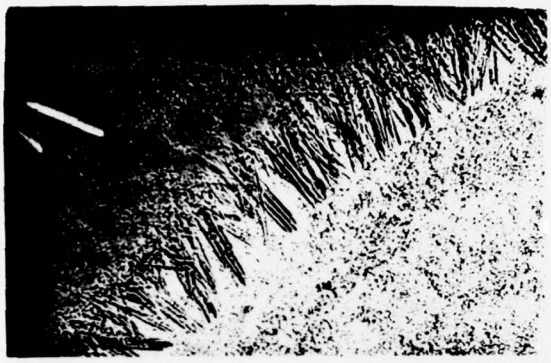


e.) Phasianidae
Numida melfagris
(Common guinea fowl)

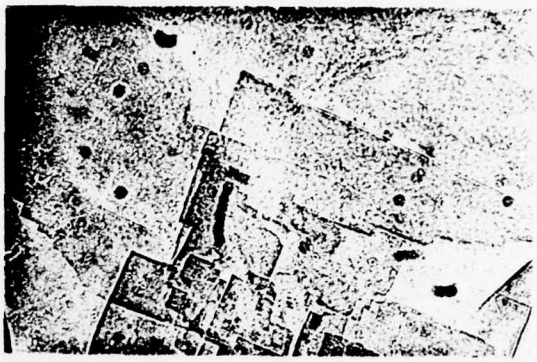


f.) Meleagrididae
Gallus
(Domestic chicken)

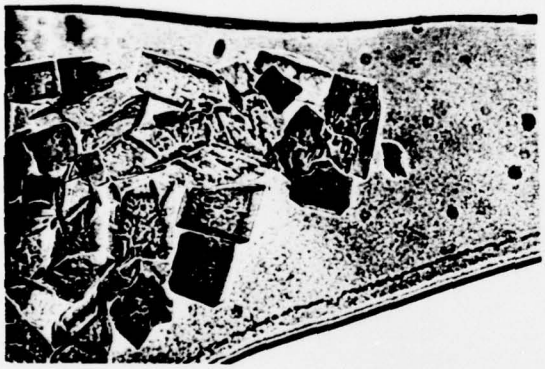
App. 61: class: AVES; order: GALLIFORMES, COLUMBIFORMES, STRIGIFORMES



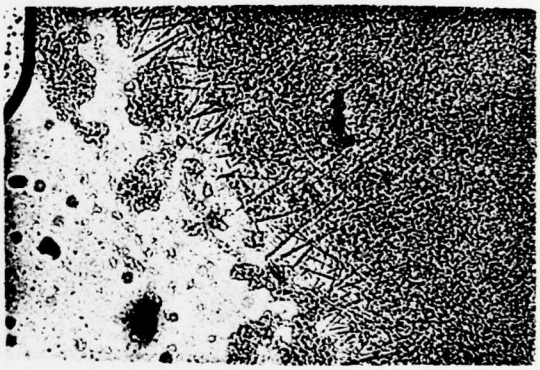
a.) Meleagrididae
Gallus
(Bantu chicken)



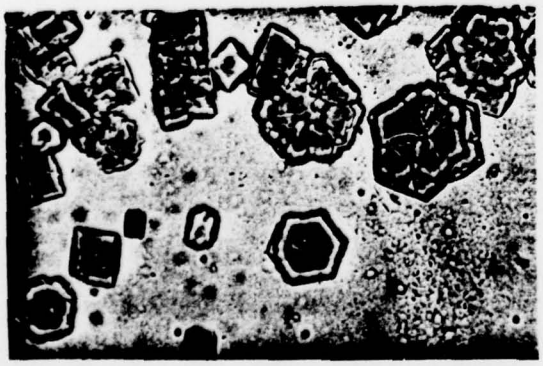
b.) Columbidae
Columbia livia
(Rock dove)



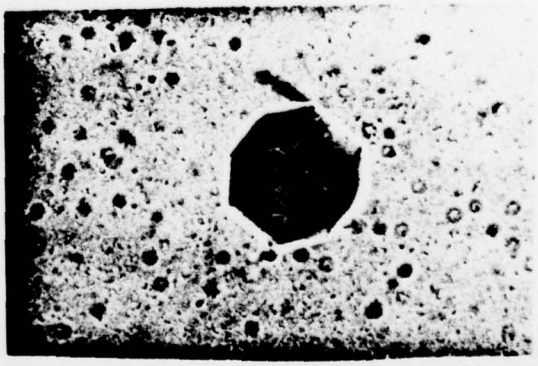
c.) Columbidae
C. livia
(Rock dove)



d.) Columbidae
C. livia
(Rock dove)

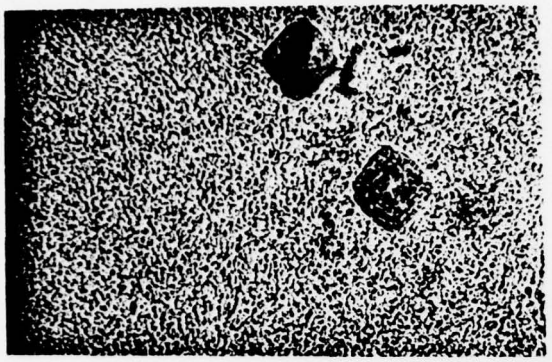


e.) Tytonidae
Tyto alba
(Common barn owl)

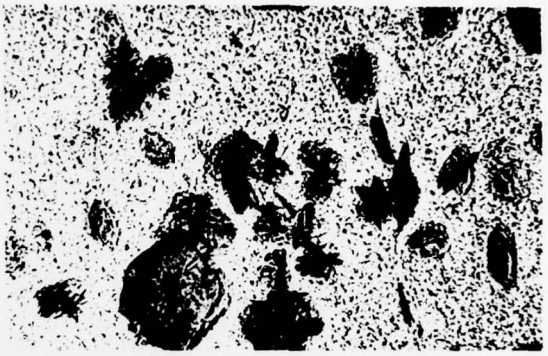


f.) Tytonidae
T. alba
(Common barn owl)

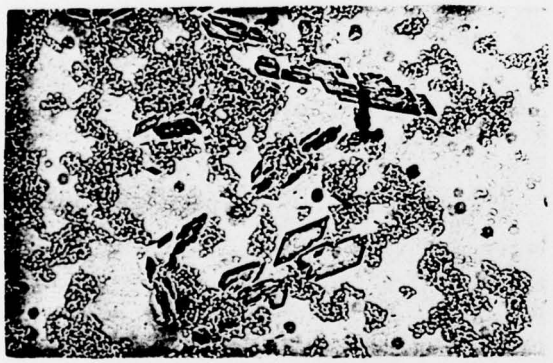
App. 62: class: AVES; order: PICIFORMES, PASSERIFORMES



a.) Picidae
Colaptes auratus
(Common flicker)

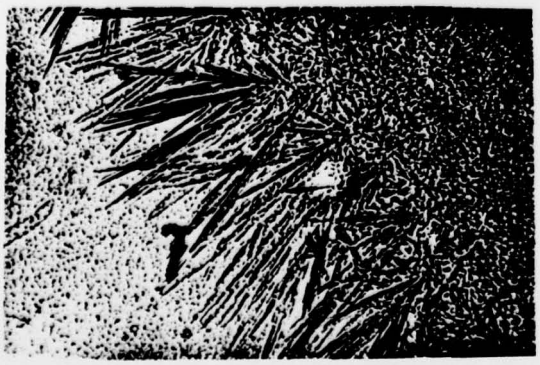


b.) Picidae
C. auratus
(Common flicker)



c.) Corridae
Corvus brachyrhynchos
(Common crow)

d.) Turdidae
Turdus migratorius
(Robin)

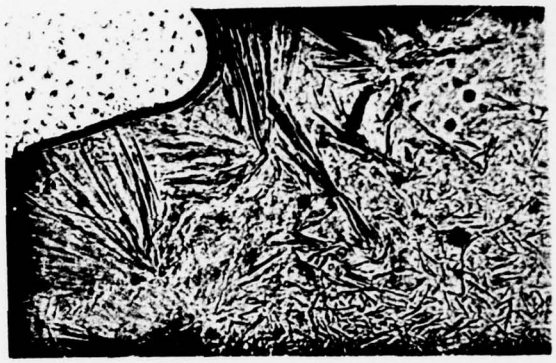


e.) Bombycillidae
Bombycilla cedrorum
(Cedar waxwing)

f.) Icteridae
Euphagus cyanocephalus
(Brewer's blackbird)

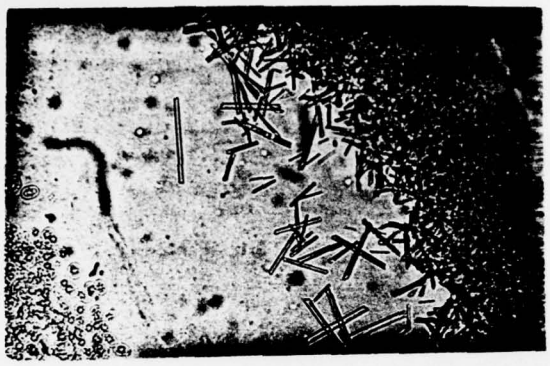
App. 63: class: AVES; order: PASSERIFORMES

a.) Sturnidae
Sturnus vulgaris
(Starling)



b.) Sturnidae
S. vulgaris
(Starling)

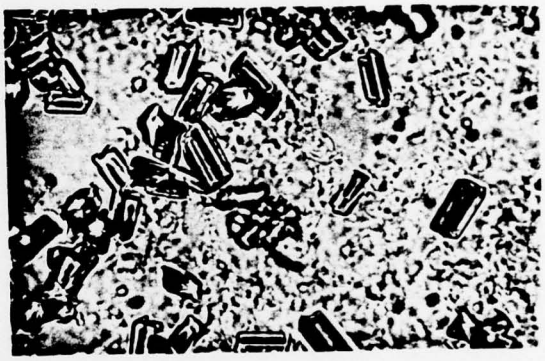
App. 64: class: REPTILIA; order: TESTUDINATA, SQUAMATA



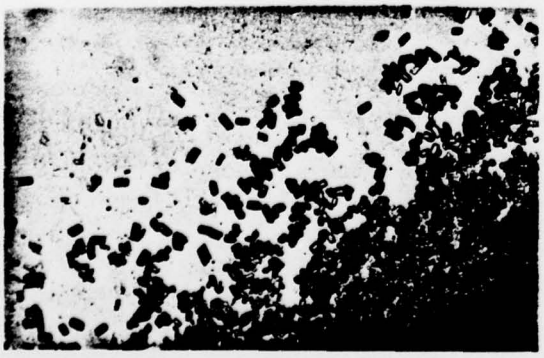
a.) Testudinidae
Pseudemys scripta elegans
(Red-eared turtle)

b.) Testudinidae
P. scripta elegans
(Red-eared turtle)

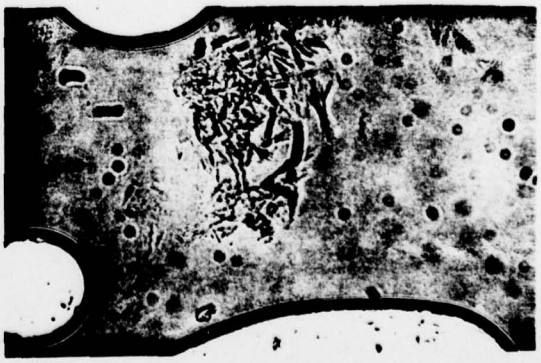
c.) Testudinidae
Geochelone elephantos
(Galapagos tortoise)



d.) Testudinidae
G. elephantos
(Galapagos tortoise)



e.) Testudinidae
G. elephantos
(Galapagos tortoise)



f.) Iguanidae
Iguana iguana
(Green iguana)

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An accurate knowledge of hosts of hematophagous arthropods is essential in studies of diseases transmitted by arthropods. Identification of blood meals of these arthropods requires a test which is sensitive enough to detect digested blood and specific enough to identify the various hosts. The study presently being reported on is concerned with a method which involves crystallization of the hemoglobin (Hb) in the blood samples from the arthropod

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midgut and comparing the crystal structure with that of known material.

Crystallization of vertebrate blood samples were more easily attained with the relatively insoluble Hb than with the more soluble ones. Techniques aimed at reducing the solubility of more soluble Hb's, buffering at human Hb's isoelectric point, were used to improve crystallization success.

The final reagents for Hb crystallization of a midgut sample from a blood engorged mosquito was to treat half of the sample with .035M ammonia oxalate, pH 6.8, in a 0.025M phosphate buffer, and the other half with .07M ammonia oxalate, pH 6.15 $\pm$ .02 in a 1.5M phosphate buffer. The high molarity buffer reagent was to ensure crystallization of cow blood meals and the low molarity reagent for most of the other mammalian blood meals.

Further studies were conducted on the variability of the crystal growth with respect to different mosquitoes and vertebrate hosts. Hb solubility and to a lesser extent, the mosquito species involved, appear to govern the success of the crystallization technique for individual vertebrate host species. Therefore, the crystallization success of any blood meal containing soluble Hb may be considerably less in some mosquitoes; conversely, with blood meals containing insoluble Hb, little difficulty is encountered regardless of the mosquito species involved.

The results of preliminary isoelectric focusing experiment with cow blood indicated that cow Hb was altered quite rapidly in the process of the blood meal digestion in the mosquito, and it is this rapid loss in concentration of the soluble Hb which plays a part in the reduction of crystallization success with the presently used reagent.

On the basis of electrophorectic studies, there appears to be no correlation between electrophoretic mobility, solubility or crystal morphology.

No changes in the electrophoretic properties of cow blood were observed even after prolonged storage (1 year). Crystallization, however, was less reliable than with fresh samples. The results of identifying multiple blood meals by the Hb method varied with different combinations of hosts. Some blood mixtures were negative; others produced one or both of the individual crystal forms; still others formed hybrid crystals.

Limited samples of known tick and bedbug blood meals were processed and identified successfully.

The accuracy of identifying unknown blood samples by the Hb technique was assessed by conducting (1) blind tests in the laboratory, and (2) identifying blood meals of field-collected mosquitoes by the precipitin and Hb tests and comparing results. The results of the blind tests were low (64 and 56 per cent). Scores for some blood meals (i.e., horse) were high (24 of 24) irrespective of time; others were high (cow, 8 of 8) only for the first 6 hours; still others were consistently low irrespective of time (man, 4 of 17). The comparison of the Hb with precipitin test is still incomplete. At this stage, the proportion of mosquito blood meals reacting to any host with the Hb method (51.6 per cent of 860) is significantly lower than the results of the precipitin test when the mammalian-negative category is excluded (97.8 per cent of 1408) or included (77.0 per cent).

Hb crystals were induced from 93% of the 170 mammalian species tested. 89.5% of the mammals tested formed crystals with the normal low molarity buffer reagent (0.035M ammonia oxalate, pH 6.8, 0.02M buffer). Only 46.7% of the 45 different bird species tested produced crystals; three of 13 reptiles tested formed Hb crystals with the normal reagent. Photomicrographs of the crystals of most vertebrates tested is catalogued according to host phylogeny and/or Hb crystal morphology.