Comparative Pathology Course May 10-12

The 3rd annual Comparative Pathology Course will be presented May 10-12 at the Armed Forces Institute of Pathology, Washington, D.C.

This course is specially designed to bring attention to disease processes in animals for which a similar entity occurs in man. Differences and similarities of pathologic lesions, as well as the biological behavior of specific entities, will be compared in animals and man.

Military and federal service employees in the medical, veterinary and other medical science fields are requested to consult respective agency regulations for appropriate application procedures. Civilian physicians, veterinarians, and allied scientists are invited to apply and will be considered on a space available basis.

Application forms to attend this course may be obtained by contacting: The Director, Armed Forces Institute of Pathology (AFIP-EDL), Washington, D.C. 20306. Completed application forms should be returned by April 12. Non-federal civilians and foreign nationals are required to submit a $75 fee, payable to the Treasurer of the United States.

MYSTERY CASE NO. 9

Mucosal surface (1) and cross section (2) of the cecum of a female Brown-eared pheasant (Crossoptilon mantchurianum). The nodules, about 2 mm. in diameter, were generally located in the submucosa.

One of the accessions contributed to the Registry of Marine Pathology: A condition of unknown etiology in the blue crab, Callinectes sapidus. The exoskeleton is raised anterodorsally, eventually breaking off and leaving an ulcer.

Marine Pathology Registry Established

A Registry of Marine Pathology has been established at the Oxford Laboratory of NOAA's Middle Atlantic Coastal Fisheries Center to solicit, catalogue and maintain accessions representative of pathology and abnormality in marine and estuarine biota.

Accessions are solicited in the following order of preference: 1) slides, photographs and publications; 2) tissue blocks; 3) fixed tissues. Qualified investigators are invited to donate suitable material to ROMP and to avail themselves of its facilities. An initial catalogue listing about 175 accessions and 90 pathogens, parasites or diseases will be available on request.

To receive catalogue, contribute accessions or be placed on the mailing list, write to Curator, Registry of Marine Pathology, Middle Atlantic Coastal Fisheries Center, Oxford, Maryland 21654.

Conference of the IAAAM

The 7th annual conference of the International Association of Aquatic Animal Medicine, will be held at the Olympic Hotel, Seattle, Washington, April 25-28. For further information about the conference, contact Dr. Jay Hyman, 37 Montebello Road, Suffern, N.Y. 10901.

New items about recent and new developments in comparative pathology, suggestions for ways to improve the Bulletin, new names for the mailing list can all be sent to the Registry of Comparative Pathology.

The Registry of Comparative Pathology is supported in part by Public Health Service Research Grant No. RR00301 from Division of Research Resources, United States Department of Health, Education and Welfare, under the auspices of Universities Associated for Research and Education in Pathology, Inc.
MALARIA IN THE OWL MONKEY

Contributed by Martin D. Young, Sc.D., Richard N. Rossan, Ph.D., and David C. Baerg, Ph.D., Gorgas Memorial Laboratory, Balboa Heights, Canal Zone.

Human Disease: Malaria

Animal Model: Aotus trivirgatus (night or owl monkey) infected with human malaria parasites.

Biologic Features: The disease in man is caused by one or more of the four species of plasmodia. These tend to be host specific in nature, as there is virtually no proof of monkeys with naturally acquired human malaria infections. Transmission is either by mosquitoes or artificially by injection of infected blood. Chills, fever, anemia, splenomegaly, hepatomegaly and prostration are common attributes of the disease in man. Deaths from fulminating P. falciparum infections occur.

We demonstrated recently that human malaria parasites, i.e., P. vivax, could be grown successfully and maintained serially in New York monkeys (1). Later, it was shown that P. falciparum (2) and then P. malariae (3) could also be grown in these monkeys. These small monkeys have many advantages as laboratory models (4).

The Aotus trivirgatus is very susceptible to P. vivax of human origin, especially when the monkey is given immunosuppressant drugs, splenectomized, or both. Once established in the Aotus, the malaria can then be passed easily to other monkeys, even to those not splenectomized or given drugs. Some strains have been maintained for over 7 years (5,6).

Comparison with human disease

P. vivax:

Prepatent periods of blood-induced infections vary more widely in monkeys than in man. The course of the infection is similar to that in man, with fewer exceptions. The primary attack may persist several weeks with the parasitemias reaching high levels and then declining. Chronicity may then occur with low level parasitemias interspersed with subpatent periods. Parasitemias tend to reach higher densities in the monkeys than in man. Also the parasitemias in the relapses may be higher than those in the primary attack.

The morphology of the parasite and its influence on the host red blood cell appear to be the same (Fig. 1).

Chills and fevers characterize the human hosts but not the monkey.

Anemia occurs in both hosts, as well as anorexia and malaise. Splenomegaly is common to both hosts.

The response of the blood-induced infections to the standard drugs appear to be similar.

Gametocytes occur but appear to be fewer in the monkey hosts. They are infective to certain types of anopheline mosquitoes but less so when the monkey is the host. Transmission by the infected mosquitoes occurs but apparently less readily when the monkey is the recipient. The resultant prepatent periods tend to be longer in monkeys (1,7).

Exoerythrocytic bodies, the stage between the sporozoite and the erythrocytic forms, occur in the liver of both human and monkey hosts.

The response to drugs of the sporozoite-induced infections in both hosts, based on limited observations, appears to be grossly similar. Relapses occur after the use of the standard 4-aminoquinoline schizontocidal drugs but cures are obtained generally after the use of the 8-aminoquinoline drugs.

There is an increase in infectivity of blood-trophozoites after continuous passage through the monkey hosts, as compared to no comparable changes during passages in the human hosts.

P. falciparum:

Virtually all of the similarities and differences listed above for P. vivax exist for P. falciparum in man and monkeys with the following additional observations.

P. falciparum induced in monkeys tends to run a parasitological course somewhat similar to that in man, although in some cases the maximum parasitemias may be extraordinarily high in monkeys (8).

One of the earliest evidences of infection in the monkey is the appearance in the peripheral blood of full-grown segments. These forms may be found frequently in the peripheral blood throughout the infection. In contrast, these forms are rarely present in the peripheral blood of man. Early appearance of the segments as the first indication of infection has rarely, if ever, been reported for man (9).

Sequestration of the parasitemias, especially those more than half grown, occurs in the internal organs in both hosts but perhaps to a lesser extent in the monkey (10).

Gametocytes are produced. Mosquitoes can be infected and can transmit the infections to man and to other monkeys (11,12).

P. falciparum appears to have a far more general infectivity to the various human races than to the different varieties or subspecies of Aotus monkeys.

Usefulness of this model: This model may be useful in solving several problems:

1. Differences in susceptibility of various geographic strains of Aotus to the same strain of human malaria, and conversely, differences in susceptibility of one geographic strain of Aotus monkey to different geographic strains of human malaria parasites;
2. Elucidation of development of homologous and heterologous immunity by strains and species of parasites of human malaria in the same monkey host;
3. Influence of intercurrent infections by other pathogenic agents upon the malaria infection;
4. Modification of the susceptibility of the monkey hosts by the use of immuno-suppressive drugs or splenectomy;
5. Factors governing gametocyte production and their infectivity to mosquitoes;
6. Fate and survival of sporozoites when injected into the monkey host;
7. Relationship of exoerythrocytic stages in internal organs to: (a) production of forms that invade the erythrocytes; (b) persistence of forms that cause relapses; (c) production of precursors of gametocytes, especially in P. falciparum malaria;
8. Factors which govern the susceptibility of mosquitoes to the gametocytes and which govern the completion of the sporogonic cycle in the mosquito and the resulting infectivity (Continued on page 4).
OF HUMAN DISEASE

DIABETES MELLITUS

Contributed by Charles F. Howard Jr., Ph.D., Section on Nutrition and Metabolic Diseases, and James L. Palotay, D.V.M., Laboratory of Pathology, Oregon Regional Primate Research Center, Beaverton, Oregon 97005.

Human disease: Diabetes mellitus

Animal model: Diabetic syndrome in Macaca nigra

Biologic Features: Severely diabetic Macaca nigra lose weight and are lethargic. Glucosuria is often found with polyuria, polydipsia and polyphagia; ketonemia and ketonuria are only occasionally present.

Table 1 lists several serum constituents, their normal ranges, and the approximate limits of abnormal concentrations.

In severely diabetic monkeys, inappropriate hyperglycemia, which begins at 125 mg/dl, often exceeds 500 mg/dl. Figure 1 depicts the findings of a representative intravenous glucose tolerance test (IV-GTT) in a normal and diabetic monkey. K* values from the IV-GTT were less than 1.0 in diabetic monkeys and generally higher than 2.0 in normal monkeys; an intermediate group was classified as borderline diabetic to indicate some alterations in glucose clearance.

The concentrations of immunoreactive insulin (IRI) were much lower in fasting diabetic than in normal monkeys (Table 1), and the response of diabetic monkeys to glucose during an IV-GTT was impaired (Fig. 1). Lipid components were increased in diabetes-prone monkeys. Hypertriglyceridemia, which was established in Macaca nigra at about 130 mg/dl, was concomitant with increased prebeta-lipoprotein. As measured on agarose gel electrophoresis, the average 10 to 20% prebeta-lipoprotein in normal mature monkeys increased to over 25% of the total lipoproteins in diabetic monkeys (1).

The fact that cholesterol was not affected by the diabetic status may be due to the animals' consumption of a commercial chow that contained less than 0.01% cholesterol. Classification as a diabetic was based mainly on abnormal ranges of hyperglycemia and impaired glucose clearance; then on IRI and triglycerides. About 50% of the almost 80 Macaca nigra that have been tested so far are normal, 35 to 40% have been classified as intermediate borderline diabetics and 10 to 15% as severely diabetic.

Pathologic features: A major pathologic change found in these diabetic animals was partial to complete hyalin infiltration of the pancreatic islets (Fig. 2). The eosinophilic hyalin material stained red with alkaline Congo red and when viewed under polarized light exhibited green birefringence. Standardized toluidine blue (STB) staining produced an orthochromatic blue color with red birefringence. The islets hyalin material reacted negatively to iodine and when viewed by electron microscopy had the fibrillar configuration of amyloid (2).

Diabetic monkeys usually had greater than 80% infiltration of amyloid and this was accompanied by a gradually increasing inability to secrete insulin. Borderline monkeys usually had partial infiltration of the islets.

Aortic atherosclerosis was most severe in diabetic monkeys (3). Normal monkeys of comparable age had slight subintimal thickening with some sudanophilic blush, and the least severely diabetic had aortic thickening and sudanophilia throughout the endothelium with a few lesions. In the most severely diabetic, 75% of the surface had both lipid and elastomuscular lesions (Fig. 3). There was fat infiltration and a

0.693 x 100 = percent glucose cleared per minute during an IV-GTT

\[ K = \frac{t_2}{t_1} \]

where \( t_1 \) = the time taken to reduce the concentration of serum glucose by one half.

TABLE I

<table>
<thead>
<tr>
<th>Serum</th>
<th>Normal range</th>
<th>Limits of abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>60-110 mg/dl</td>
<td>&gt; 125 mg/dl</td>
</tr>
<tr>
<td>K (from IV-GTT)</td>
<td>&gt; 2.0</td>
<td>&lt; 1.0% glucose decrease/min</td>
</tr>
<tr>
<td>Insulin</td>
<td>25-35 μIU/ml</td>
<td>&lt; 20 μIU/ml</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>85-110 mg/dl</td>
<td>&gt; 130 mg/dl</td>
</tr>
<tr>
<td>Prebeta-lipoprotein</td>
<td>10-20</td>
<td>&gt; 25% of the total lipoprotein</td>
</tr>
</tbody>
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Fig. 1. Representative IV-GTT from a normal (left) and diabetic monkey (right). In the normal test, the glucose load caused a rapid response of insulin and the glucose was rapidly cleared (K = 2.15). The diabetic monkey failed to respond with adequate insulin and the slow clearance of glucose gave a K = 0.7.

Fig. 2. Islet of Langerhans of a diabetic monkey with extensive amyloid infiltration. Note the remaining, apparently viable cells capable of secreting some insulin. 250X; hematoxylin and eosin.

Fig. 3. Aorta from a diabetic Monkey. Sudan IV stain reveals extensive lipid lesions.

(Continued on page 4)
Diabetes Mellitus ............... (Continued from page 3)

femoral muscle increased from an average of 700 A in normal to
840 A in diabetic monkeys (20%).

No necrosupnten dilatations have been observed in
trypsin-digested preparations of retinal vasculatures. Prelimi-
nary studies in kidneys suggest early diabetic glomerulone-
sclerosis with subtle diffuse thickening of the mesangial area
and capillary basement membrane.

Under a prolonged regimen of hyperglycemia and insulin
depression, a few monkeys have developed cataracts which
are likewise commonly observed in other experimental animal
models with hyperglycemia.

Comparison with Human Disease: In a significant percentage
of these monkeys, inappropriate hyperglycemia and impaired
IV-GTT bear a striking resemblance to human diabetes
mellitus. Triglycerides are significantly increased in diabetic
monkeys but less so than in diabetic humans, probably
because of the low fat content ingested by the monkeys.
Amyloid infiltration into the islets of Langerhans, together
with the loss of beta cells and insulin secretion, resemble
an intense manifestation of a particular aspect that can appear
with aging and with the onset of human diabetes. This
amyloidosis is islet specific in Macaca nigra and does not
infiltrate into other tissues. Similar infiltration has been seen
in other nonhuman primate species (5,6).

The gradual inability to secrete insulin and to control

Malaria in Owl Monkey .......(Continued from page 2)

of the sporozoites:


Availability: In the past, owl monkeys have been exported
from several South American countries (Peru, Colombia,
Brazil). At the time of this writing, the governments of all of
these countries had placed an embargo on such exports.

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that originated from the Gorgias Memorial Laboratory was
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and DADA 17-68-C-8137. This contribution number 1243
to the Army Research Program on malaria.

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Plasmodium falciparum in the organs of Aedes aegypti nectar


falciparum from monkey to monkey by the bite of infected

glucose and lipid metabolism places the monkey in a meta-
abolically stressed state which over a period of years fosters
the development of such secondary complications as athere-
sclerosis, cataracts, and increased thickness of the basal lamina
of muscle capillaries.

Availability: Macaca nigra inhabit the northeast corner of the
Celebes Island (Celebes), a part of the Indonesian archipelago.
The large colony at the Oregon Primate Center is generally
not available to other investigators, but other individuals or troops
are kept at other institutions and zoos in the United States and
throughout the world. Although they are becoming commer-
cially available through importers, a word of caution: little
is known about the social and breeding habits of these
monkeys in their natural habitat, and it would be wise to
proceed with great care and extreme caution in their importa-
tion to avoid endangering the species or forcing them into the
rare animals category.

Acknowledgment: This publication from the Oregon Regional
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RR 00163, both from the Division of Research Resources,
National Institutes of Health. This work was also supported by
funds from the Kroc Foundation and PLS grant HL-16661.

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Macaca cyclopis and Mandulitus leucophus: case reports. Lab.

MYSTERY CASE NO. 9 – ANSWER

Diagnosis: Heterakis nodularis and nodular granulomas due to

Heterakis isolonica.

Nodules were generally composed of various types of cells,
most of which were fibroblasts, macrophages and lympho-
cytes. Within the nodules were nematodes which were
identified as Heterakis isolonica (see photograph, X14).

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