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ALASKA DEPARTMENT OF FISH AND GAME

JUNEAU, ALASKA

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DISEASE AND PARASITE STUDIES

by

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STUDY PLAN SEGMENT REPORT

FEDERAL AID IN WILDLIFE RESTORATION

State: Alaska

PROJECT NO.: W-17-2 TITLE: Big Game Investigations

STUDY PLAN: R TITLE: Disease and Parasite Investigations

JOB NOS.: 18.1 TITLE: Caribou and Reindeer

18.2 TITLE: Sheep

18.3 TITLE: Alternate Host Species

18.4 TITLE: Nutritional Physiology of Caribou and Moose

PERIOD COVERED: January 1, 1969 to December 31, 1969

ABSTRACT

Job No. 18.1: Caribou and Reindeer

Comparison of the standard agglutination and complement fixation serological tests for antibodies against Brucella suis biotype 4 in caribou suggests that the latter test is the more sensitive.

Infections of rangiferine brucellosis in sled dogs have been confirmed by isolation of the organism from a member of an infected team. Serological data on wolves suggest that they also may carry the infection.

Lack of even one serological, brucella reactor among fifteen cows with retained placental material collected on the Arctic calving grounds suggests that this condition may have a non-brucellar etiology.

Job No. 18.2: Sheep

Efforts are underway to develop a technique for rating the degree of severity of "lumpy jaw" and/or necrotic stomatitis infections of the mandibles of Dall sheep.

Adult specimens of a third kind of lungworm (i.e. Muellarius sp.) from Alaskan Dall sheep are not yet available for study.

Job No. 18.3: Alternate Host Species

Two serotypes of Leptospira have been detected in eight of fifty-one moose tested.

Four "suspicious" titres for brucella have been detected among 15 sera from bison taken by hunters from the Big Delta herd. Two of eight bison from this herd yielded titres for a serotype of Leptospira.

One hundred and thirty-five lynx from interior Alaska were commonly infected by one or more individuals of five common species of worms parasitic in lynx in Alaska.

Two muskox died of pneumonia and probably other complicating factors soon after being transplanted to the vicinity of Barter Island.

Job No. 18.4: Nutrition Physiology of Caribou and Moose

The dry weight of femur marrow samples from caribou is an accurate measure of fat content at fat levels of 20% or more. At fat levels less than 20% the dry weight value may be corrected by subtracting the average non-fat-residue value from the specific dry weight level involved.

Femur marrow fat levels of about 40% or less appear to show some correlation with the overall condition of caribou. Femur marrow fat levels of adult male caribou may be more sensitive to range condition and climate or other stress factors than marrow fat in adult females.

STUDY PLAN SEGMENT REPORT

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OBJECTIVES

To determine the incidence and distribution of potential pathogens in Alaskan wildlife species and alternate or reservoir hosts.

To determine whenever possible or practical the extent that such organisms may contribute to mortality or lowered productivity or economic value of affected wildlife species.

To determine the extent that wildlife pathogens depreciate the value of game animals for use as food by humans or may be a threat to domestic animal industry.

To develop useful techniques for biochemically evaluating the nutritional state of wild ungulates and the nutritional quality of their forage.

Job No. 18.1: Caribou and Reindeer

PROCEDURES

At the present time the primary effort in rangiferine disease studies is focused on the long term study of brucellosis in caribou. In this respect we are continuing our close cooperation with the Animal Disease Eradication Division, U. S. Department of Agriculture who is monitoring the disease in reindeer. In these studies the following specific procedures are emphasized.

1. Serological surveillance of brucellosis prevalence in major caribou herds particularly those in the Nelchina and Arctic areas.

2. Confirmation by isolation of suspected brucellar infections.

3. Serological studies on potential reservoir host species.

4. Aerial surveillance of the occurrence of animals displaying gross symptoms (i.e. limping, retention of afterbirth) of brucellosis during calving.

5. Surveillance from the ground of concentrations of animals during the spring and fall migrations through Anaktuvuk Pass in the Arctic to detect and collect specific animals for bacteriological and/or other studies.

6. Routine autopsies of animals taken for subsistence purposes by native or sport hunters or specifically for the purposes of various scientific studies (e.g. radiation studies, disease and parasite studies, etc.).

7. Examination of specimens submitted to our laboratory by the public.

8. Preparation of a definitive bibliography on the "Diseases, Parasites, and Disorders of Caribou and Reindeer."

9. Publication of data at suitable intervals.

FINDINGS

A. Serological Studies: Brucellosis

1. Caribou

Data on the current prevalence of brucellosis reactors in caribou are presented in Table 1. While data are too meager for any one herd to be able to confidently compare prevalence rates for 1969 with other years, a comparison of the results obtained by the different test methods is interesting. Two conclusions may be tentatively formed. The complement fixation test regularly gives higher test reactions than the agglutination procedure and it may also detect infections that either the agglutination or "USDA card" test failed to detect at all. The latter conclusion assumes that a CF titre of 1:20 is indicative of infection. An agglutination titre of 1:20 is usually only considered to indicate a "suspect" animal that should be retested later. The greater sensitivity and specificity of the CF test probably justify giving

more weight to this test than the simpler, less sensitive agglutination test. This possibility has been previously considered (Neiland et al., 1968).

2. Canines

The possibility that wild or domestic canines contract rangiferine brucellosis from eating infected meat and/or offal was raised some time ago (Neiland, loc. cit.). The first evidence that this does occur was obtained at Anaktuvuk Pass in May, 1967, when two surviving members of a dog team yielded agglutination titres of 2+, 1:160 and 2+, 1:640.

More recently, additional evidence has been obtained which proves the infection of sled dogs and indicates infection of wolves. The evidence pertaining to wolves can be quickly summarized by noting that three of seven wolves taken near Anaktuvuk Pass in early 1969 yielded complement fixation titres of 1:20, 1:40 and 1:160 respectively, the latter also giving an agglutination titre of 1:160. The additional data on sled dogs are worthy of detailed consideration.

An infected dog team was discovered at Kobuk in May 1969. The serological data on this and several other teams owned by residents of the village are shown in Table 2. Lucy, the first infected dog detected, was brought into the laboratory for sacrifice when the July sera were collected. The results of the ante mortem examination are shown in Table 3 and those of the bacteriological examination are shown in Table 4. Several tentative conclusions may be drawn at this time from the still limited data:

1. Infections of Brucella suis biotype 4 in sled dogs is probably common and is contracted from infected meat or offal used as dog food in many native villages.

2. The serological response to the infection may have a fluctuating character, perhaps depending in part upon the nutritional status of the animal.

3. The disease may not produce obvious clinical symptoms.

4. Wild canines are probably also commonly infected in areas in which enzootic rangiferine brucellosis occurs.

Whether infected canines are also sources of infection for other animals remains to be seen. Other biotypes of Brucella are known to be transmissable from one dog to another or to other hosts.

B. Calving Ground Studies

The occurrence of the "placental retention" condition in animals on the Arctic calving grounds in 1969 is shown in comparison with previous years in Table 5. The significance of the data for 1969 depends upon the fact that none of the 15 animals displaying the condition which were collected yielded serological or other evidence of involvement with brucellosis. Only 6 of the 41 animals showing placental retention were unaccompanied by calves in contrast with 1968 when 58% of such animals were without calf. The weather was noticeably less severe in 1969.

Table 1. Prevalence of brucella reactors in caribou, 1969.

Herd	Specimen Number	Serological Results				
		Negative	Positive	Complement Fixation Test	Agglutination Test	USDA Card Test
Arctic	A 54369		+	4+, 1:160	4+, 1:40	+
	A 50596		+	2+, 1:320	2+, 1:40	+
	A 50604		+	4+, 1:80	Neg.	Neg.
	A 50607		+	4+, 1:20	Neg.	Neg.
	A 50609		+	4+, 1:20	Neg.	Neg.
	A 50611		+	4+, 1:640	2+, 1:40	+
	A 50620		+	2+, 1:320	4+, 1:160	+
	A 50626		+	2+, 1:40	2+, 1:40	---
	A 50637		+	4+, 1:320	2+, 1:80	---
	Total:	60	9	---	---	---
Nelchina	---	5	---	---	---	---
Adak	---	4	---	---	---	---
Alaska Range	# 7876-7877		+	4+, 1:40	2+, 1:20	---
	Total:	57	1	---	---	---
Alaska Peninsula		7	---	---	---	---

Table 2. The occurrence and persistence of serological titres associated with rangiferine brucellosis in sled dogs.

		Titres								
		May		July			September		December	
	Name of Dog	Aggl. Test	Comp. Fix. Test	Aggl. Test	Comp. Fix. Test	USDA Card Test	Aggl. Test	Comp. Fix. Test	Aggl. Test	Comp. Fix. Test
Infected Team[1]	Beaver	--	--	Neg.	Neg.	Neg.	Neg.	1:20	Neg.	Neg.
	Fannie	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Harry	--	--	Neg.	1:20	Neg.	1:320	1:640	Neg.	?
	Jumbo	--	--	1:640	1:640	Pos.	1:20	1:20	1:160	1:160
	King	Neg.	Neg.	1:160	1:10	Pos.	Neg.	Neg.	--	--
	Lucy[2]	1:2560	1:640	1:640	1:1280	Pos.	--	--	--	--
	Mila	Neg.	Neg.	Neg.	1:10	Neg.	Neg.	Neg.	--	--
	Moose	--	--	1:640	1:1280	Pos.	1:80	1:10	1:160	1:40
	Nellie	--	--	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Wendy	Neg.	Neg.	Neg.	Neg.	Neg.	1:160	1:80	Neg.	Neg.
Other Dog Teams[1]		4 Teams 18 Dogs Negative	--	--	--	--	2 Teams 10 Dogs Negative	2 Teams 10 Dogs Negative	--	--

[1] It was not practical to attempt to take serum from all members of some teams each time.

[2] Sacrificed at time of July sera collection. Brucella suis Type 4 isolated from several tissues (See Table 4).

Table 3. Ante mortem condition of a sled dog infected with Brucella suis biotype 4.

Observation	Value	Comment
Temperature (anal)	101.8 F°	Normal
Heart rate	108/minute	"
Lung sound	---	"
Physical exam in general	---	"
Blood count:		
PCV	43	"
Hemoglobin	14 gm.%	"
WBC	14,500	"
Differential count:		
Lymphocytes	26%	"
Monocytes	6%	"
Eosinophils	20%	"
Segmenters	48%	"

Table 4. Occurrence of Brucella suis type 4 in selected tissues of a sled dog.

Tissue	Results[1]
Mandibular lymph nodes	+++
Mesenteric lymph nodes	+++
Popliteal lymph nodes	+++
Subscapular lymph nodes	++
Mediastinal lymph nodes	_[2]
Spleen	+
Liver	_[2]
Lung	_[2]
Heart muscle	_[2]

[1] Numbers of colonies observed on plate cultures were scored as follows: 1-10, +; 10-100, ++; over 100, +++.

[2] Two or more attempts to recover organisms in culture were made.

Table 5. Prevalence of various abnormal animals in the Arctic calving herd, 1966-69.

Year	Condition	Sample Size	Number	Percent
1966	Placental retention and/or hemorrhagic perineum	2058[1]	34	1.6
1967	" "	2832[1]	16	0.45
1968	" "	2010[1]	52	2.6
1969	" "	4357[1]	41	0.94
1966	Limpers	2058 plus[2]	14	0.7
1967	"	2832 plus[2]	14	0.5
1968	"	2010 plus[2]	7	0.3
1969	"	4357 plus[2]	19	0.4

[1] Includes only animals with calves or showing the condition.

[2] Includes all animals examined for placental retention plus hundreds(?) of others.

C. Studies on Migratory Concentrations: Anaktuvuk Pass

During the spring movement north through the pass, we had a chance to examine 25 caribou. The usual common parasites were encountered in moderate infestations. Of particular interest were two individuals each of which had single carpal hygromas which caused severe limping. The condition can be purely traumatic in origin but can become secondarily infected by Brucella or due solely to this pathogen. Brucellar hygromas have been reported previously in Alaskan and Siberian species of Rangifer.

D. Bibliographic Studies and Publications

The bibliography on the parasites, diseases and disorders of reindeer and caribou has been completed as part 1 of a series on wild ruminants of the northern hemisphere. The bibliography contains about 750 titles of which about one-third are now available as reprints, etc. in our files. It is now planned that this bibliographic series will be issued as part of a new technical series published by our department.

A report was made on our canine brucellosis studies to the Annual Brucellosis Research Conference as an invited paper. An abbreviated form of this paper was offered to the "Journal of Wildlife Diseases" for publication. This evidently is the first publication to present information on a canine infection of Brucella suis biotype 4.

A publication on parasites of caribou is still in preparation.

Job No. 18.2: Sheep

PROCEDURES

Studies on the diseases and parasites of sheep will be carried out and published jointly with members of the sheep work plan. The procedures we will employ may be summarized as follows:

1. The primary emphasis of our studies will be focused on several study areas now being selected.

2. All animals collected for studies on reproduction, population dynamics, nutrition, etc. will be routinely autopsied in the field. Some organ systems or other selected materials will be brought into the laboratory for closed inspection in those instances where an adequate examination cannot be accomplished under field conditions.

3. Specimens from hunter-killed sheep will be handled as they come in on a volunteer basis or solicited from the public whenever situations arise which require large numbers of samples (e.g. jaws).

4. Fresh fecal pellet samples will be collected on the study areas at intervals in order to follow changes in intensities of parasitic infections. These will also be collected in other areas as time allows.

5. Routine bacteriological, parasitological, etc. methods will be employed in the laboratory to evaluate specimens that come to hand. Selected material will be referred to specialists for histopathology.

6. A definitive bibliography on the parasites, diseases and disorders of wild sheep will be prepared and kept up-to-date.

7. Significant research accomplishments will be published at irregular intervals.

FINDINGS

A. Field Studies

Several years ago the larvae of a genus of lungworms (Muellarius sp.?) previously unknown in Dall sheep were found in fecal pellets collected in the Wrangell Mountains. More recently larvae of this same genus of lungworms have been commonly found in bighorn sheep in British Columbia. The adult stage of this parasite, which is required for complete identification, has not yet been collected here or elsewhere in North American wild sheep. The adult worm can only be obtained by tediously dissecting it from fresh, infected lungs. Accordingly, it was decided that we should embark on a modest program in the Wrangell Mountains involving the collection of old ewes for parasitological examination and other life history specimens. It was only possible to make one field trip well in advance of the regular hunting season during 1969 and because of inclement weather and other complicating factors, only one ewe was collected. Lungs and other organs and specimens were taken back to base camp where microscopic examination failed to reveal adult lungworms. We hope to collect several old ewes in late June and early July, 1970, and continue our search for adults of Muellarius.

B. "Lumpy Jaw" Studies

Several years ago we initiated Alaska-wide studies on "lumpy jaw." This condition in other ruminants is caused by a species of the fungal genus Actinomyces. It is assumed that the "lumpy jaw" condition widely found in North American sheep and some other wild ruminants is also caused by the same pathogen(s). In any event the condition in individual Dall sheep may reach what appears to be severe proportions (e.g. loss of all molariform teeth and severe erosion of one or both mandibles). The condition has also been seen occasionally in Alaskan caribou in a seemingly milder form. In any event, in order to quantitatively compare prevalence and severity among different sheep herds or ranges, it was evident that we would have to develop some method of evaluating the relative severity of the condition in specimens brought in by hunters or removed from intact heads brought into local taxidermy shops. When we first initiated the project a concerted campaign was made to encourage sheep hunters and guides to bring in sheep jaws and pellet samples. The response was very disappointing and because of lack of manpower to prosecute other means of obtaining specimens, the project was inactive during the next several years. During the past year or so the re-establishment of a concerted sheep study and the placement of new area biologists in key locations has resulted in the collection of 50 pairs and/or individual mandibles for study.

In order to record the severity of the lumpy jaw condition with reasonable accuracy and in a way which will allow suitable comparison of specimens, it was decided that individual records must portray both the quality and quantity of the observed condition. Two devices are being developed to accomplish these functions. In order to make a permanent record of the condition of a pair of mandibles, a jaw data sheet showing a lateral view of both mandibles and an end view of the incisors is filled out. Fig. 1 shows a typical case of lumpy jaw. The teeth that are blacked out are missing. The teeth that are cross-hatched are protruding beyond the normal tooth-line and are generally loose. Swollen areas of the mandible are outlined and cross-hatched, and fistulous abscesses are blacked out. Stippling of swollen areas (not shown in Fig. 1) indicates developing porosity of affected areas. Receding areas of the gum line are also outlined. To facilitate comparison of the prevalence and severity of the condition in various sheep herds, a numerical "Index of Severity" must be assigned each case. Index of Severity (I. S.) scores will be of greatest value if they accurately portray the degree of functional impairment resulting from the presence of the condition. However, one should keep in mind that for purposes of comparing relative prevalence and apparent severity of the condition among several herds, some lack of functional accuracy is quite acceptable. A common degree of inaccuracy will cancel out in the comparisons. Only when one must finally attempt to evaluate the influence of lumpy jaw on animal welfare, must one strive for functional accuracy of I. S. scores. One way in which we expect to approach this problem is by comparing the severity of lumpy jaw and various other biological characteristics of animals captured (sometimes repeatedly) at the salt lick in our Dry Creek study area. In any event, we have not yet developed a numerical scoring system. However, it is quite clear that the loss of M-1 which commonly occurs has much greater functional significance than the loss of PM-2 or slight swelling of the mandible. Since we do not have nearly enough specimens to make meaningful comparisons, suffice it to say that 32 of the 50 pairs or individual mandibles that were obtained in 1969 showed evidence of almost negligible to seemingly severe necrotic stomatitis (i.e. periodontitis) and loss of teeth, and/or lumpy jaw (i.e. osteomyelitis of the mandible). Because the two phrases (i. e. necrotic stomatitis and lumpy jaw) are often mistakenly used interchangeably, it is worthwhile noting that the former refers to inflammation of the soft tissues of the mouth, while the latter refers specifically to infectious changes of a bony structure, the mandible. The organism(s) causing these conditions in dall sheep have not been isolated and it is not clear whether one or more causative agents (i.e. Actinomyces bovis and Actinobacillus lignieresii) are responsible for lumpy jaw and necrotic stomatitis, respectively, in domestic animals.

C. Publications

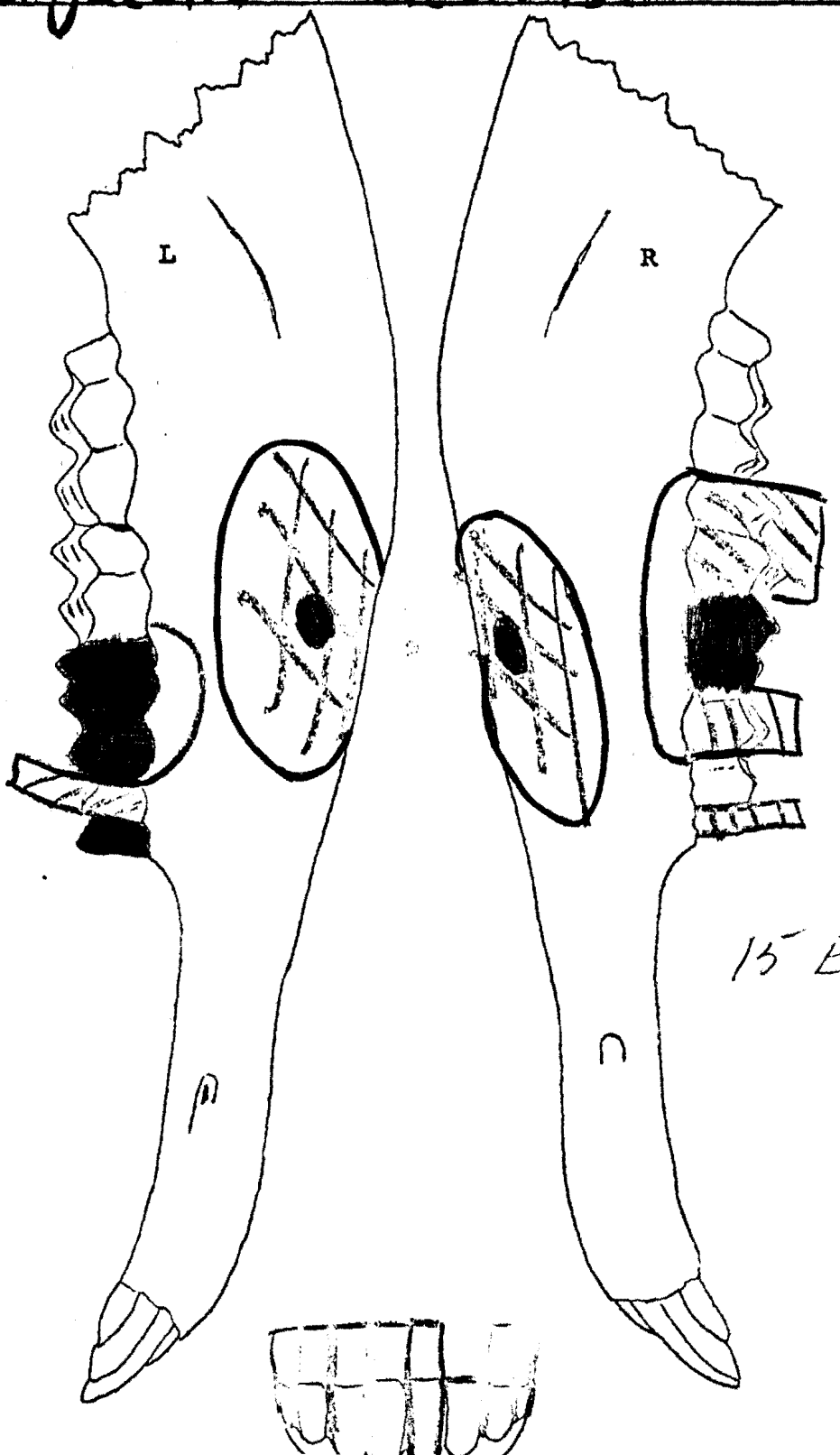
As soon as adult specimens of the lung-worm with Muellarius-like larvae are obtained we will work up the material for publication.

We will not likely have enough data on lumpy jaw and associated factors for several years to come. We may publish our technique for evaluating the material sooner.

FIGURE 1. JAW DATA SHEET

Accession Number: 1580

1. Species Dall
2. Sex ♂
3. Age
4. Date 8/10/63
5. Locality Stuver CR., Wrangells
6. Collector Atwell
7. Remarks both mandibles swollen adjacent to lesions.



15 Enamel

A preliminary contribution toward a bibliography of the parasites, diseases and disorders of wild sheep, genus Ovis, and other related mountain game was finished last spring and presented to the 2nd meeting of the Northern Sheep Council in March.

Job No. 18.3: Alternate Host Species

PROCEDURES

Field collections of host species are conducted largely in conjunction with other investigations. Members of all Divisions of the Department, as well as the public and other state or federal organizations, have been requested to send in suspected pathological specimens or in certain instances to obtain material of special interest. Such material will be handled in our laboratory, or in some cases referred to other specialists for diagnosis. At the present time special emphasis is being placed on the following host species:

1. Moose

The success of the long-term, penned-moose nutritional studies on the Kenai Peninsula will, in part, depend on an adequate knowledge of the levels of parasitism in the experimental animals. Because preliminary state-wide studies already clearly demonstrate several differences in kinds and/or numbers of parasites in moose in different areas, one can only conclude that the moose pen area may also exhibit its own peculiarities in this regard.

Because moose are probably the most sought-after species and are of great importance to all subsistence hunters, we will continue to get a variety of material from hunters and during specific life history studies by our moose work plan biologists.

2. Bison

The frequent close association between bison and domestic animals in the Big Delta farming area is one of the few instances in Alaska in which wildlife may be a potential source of diseases for domestic animals. Accordingly, it is important that, whenever bison are available for examination (e.g. controlled hunts, road kills, transplants, etc.), appropriate studies (e.g. autopsy, collection of blood samples, etc.) be made.

3. Blacktail Deer

In the Southeast Alaska and Prince William Sound areas blacktail deer are an important big game species for both sporting and subsistance purposes. For this reason we are continuing our studies on this species, in progress since 1960, but only on a limited basis. We still know relatively little about the parasites and diseases of the blacktail in many areas of the state (e.g. Kodiak, the extreme southern or western parts of Southeast Alaska).

4. Carnivores

At present we are soliciting carcasses of wolves, coyotes and wolverine taken in Southeast Alaska. There are no published data on the parasites of these predatory species in that area. However, they (wolves and coyotes?) are involved in the transmission of parasites to blacktail deer and in one instance (i.e. Echinococcus) also a human parasite. These studies were initiated in 1960 and will be continued until adequate sampling has been accomplished.

In conjunction with the lynx life history study, we have undertaken to study the levels of parasitism in this species in central Alaska from one population high to another. Relatively little is known about the parasites of lynx anywhere. We have an unusual opportunity to get quantitative as well as qualitative information.

Sled dogs, though not a wild species, nevertheless in many cases exist under semi-wild conditions. For example, in many places they commonly are fed uncooked parts of game animals which are the normal prey of wolves. Accordingly, it is possible to obtain some insight into the question of whether wolves suffer infectious diseases transmitted to them from their prey species by studying the diseases of sled dogs which are fed uncooked game. There is added interest when such diseases are also transmissable to man.

5. Marine Mammals

Many native Alaskans subsist to a large extent on marine mammals. Some marine species are also important furbearers or trophy animals. Since 1960 we have engaged in casual investigations on the parasites of a variety of marine mammals from Alaskan waters. We have also cooperated with other agencies engaged in biological studies on these species in Alaskan or other waters.

6. Miscellaneous Host Species

All species of fish and game which are prey species for various predators harbor parasites peculiar to themselves, but also the intermediate stages of parasites which mature in the predators which feed on them. In some instances these intermediate (larval) stages are of greater importance to man or beast than are those parasites peculiar only to prey species. For example, the larval stages of various tapeworms which mature in fish-eating birds or fish, may render desirable food species of fish unfit for human consumption. Some of these may occasionally infect man when partly cooked fish are eaten as is the habit of some native or non-native groups. Most, if not all, of our small game or sportfish species may be involved in these detracting host-parasite relationships. Many of the inquiries we get from the public regarding the palatability of presumably infected wildlife involve these host species. We will continue to deal with this kind of material on a casual, time-available basis.

Much of the material to be covered in a pamphlet on the "Common Diseases and Parasites of Fish and Game", now being prepared for general distribution, will involve parasites of various sportfish and small game commonly encountered by sportsmen.

FINDINGS

1. Moose

A. Parasites

Because of cancellation of anterless hunts in the Matanuska Valley, our plan to obtain an additional set of data on the prevalence of common parasites was stalled. This information was deemed necessary to be used in conjunction with a considerable mass of data obtained during earlier hunts in preparing a meaningful publication on hydatid disease.

We anticipate that we will be able to get the additional data we require during antlerless hunts this coming year.

Several examples of parasitic infestations came to hand from hunter-killed animals that were of particular interest. A moose taken along the Salcha River showed a very heavy infestation of the heart by cysticerci of Taenia krabbei. By actual count, at least 740 cysts were present. The animal was apparently in good condition otherwise and there was a heavy coronary band of fat. Two sets of specimens infested with filarial worms came in from the lower Yukon River and Taylor Highway, respectively. The "lower Yukon" material consisted of what appeared to be highly calcified specimens of Setaria sp. on the surface of the liver and peritoneal lining of the body cavity. The "Taylor Highway" material consisted of numerous specimens of another filarial worm, possibly Wehrdikmansia sp., found encysted in the subcutaneous connective tissue of a lower leg. About half of these were calcified. A few good specimens for study were tediously dissected free from the connective tissue in which they were intricately entwined.

B. Serology

A limited amount of serological data on brucellosis and leptospirosis was obtained during 1969. Thirty-seven moose taken on Fort Richardson were all negative to both the agglutination and complement fixation tests for brucellosis.

Fifty-one serum samples, including the Fort Richardson specimens and various other sera collected elsewhere, were tested for evidence of leptospiral infections by Dr. Daniel Trainer, University of Wisconsin. Seven leptospiral serotypes were checked, including Leptospira pomona, L. icterohaemorragiae, L. Hardjo, L. autumnalis, L. hebdomadis, L. grippotyphosa and L. pyrogenes. These are all more or less common serotypes widely distributed elsewhere in the world. Eight of the 51 samples showed titres of 1:100: 7 to L. icterohemorragiae and 1 to L. pomona. Without other kinds of evidence it is not possible to make any significant analysis of these data. Suffice it to say that McGowan et al (1963) reported what they thought to be the first evidence of natural leptospiral infections in moose. Four of 90 moose taken in Ontario yielded positive titres for Leptospira pomona, a serotype commonly found in white-tail deer in Ontario and other areas in eastern North America. Because of the aquatic habits of moose and because leptospiral infections are frequently transmitted by water contaminated with leptospira-bearing urine,

moose are likely hosts for leptospirosis. Because leptospirosis is principally a disease of rodents, it may be rewarding to collect sera from beaver, muskrats or other rodents in areas where moose are abundant.

C. Publications

"A Bibliography of the Parasites, Diseases and Disorders of Wild Ruminants of the Northern Hemisphere, Part II. Moose, Genus Alces." was completed. This is to become part of a new serial, technical publication to be put out by our department.

2. Bison

A. Parasites

One of fifteen sets of lungs taken from hunter-killed bison at Big Delta, September, 1969, yielded a single specimen of Dictyocaulus sp. This low prevalence of lungworms in the "Big Delta" herd in 1969 and during the past few years is in sharp contrast with the situation in 1961. At that time two-thirds of fifty animals taken by hunters during our first hunt had one or both lungs lightly or moderately infected. At that time there were evidently more bison and they were more concentrated in the farming area during more of the year. Whether conditions favoring bison to bison or cattle to bison transmission of lungworms were individually or collectively important is not known. Perhaps factors favoring survival of the free-living, infective larvae were most important in encouraging a high prevalence rate in 1961 and preceding (?) years.

B. Serology

A substantial number of bison serologically tested for brucellosis during the preceding years has always yielded completely negative results. For the first time, animals taken in 1968 (results not available for last year's report) and 1969 have yielded test results which may be termed "suspicious." The currently available data on serological tests for brucellosis in bison taken in 1968 and 1969 are shown in Table 6. In addition two of eight sera from the 1968 Big Delta collection gave titres of 1:100 against Leptospira icterohemorragiae antigen. The titres for both diseases suggest that we should maintain close surveillance on the Big Delta bison herd and never miss a chance to take sera for testing. If we encounter high titres we should make every effort to get materials suitable for attempts to isolate the organism(s). For the time being we can only conclude that the likelihood of brucellar and leptospiral infections in bison now appears somewhat greater.

3. Blacktail Deer

I had very limited field experience with blacktail deer in 1969. In February I accompanied Mr. Jack Alexander out to Whale Island where we collected two deer before weather forced us back to Kodiak. The only parasites encountered were light infestations of trichostrongylids in the abomasa of both animals. One animal, a buck, was in extremely poor condition with only a slight amount of fat showing in the coronary band of the heart. The other animal, a doe, was in comparatively good condition.

Table 6. Results of serological testing of bison, 1968 and 1969.

Herd	Date	Number Tested	Number Negative	Serological Titres Observed[1]	
				Agglutination	Complement Fixation
Big Delta	1968	9	6	+ 1:25, I - 1:50	Negative
				I 1:25	"
				I 1:25	"
Big Delta	1969	15	11	+ 1:40	Negative
				+ 1:20	"
				+ 1:20	"
				+ 1:20	"
Copper River	1969	11	11	Negative	Negative

[1] I = incomplete reaction.

The preliminary search for references on the parasites, diseases and disorders of deer, genus Odocoileus, was finished. These will comprise Part IV of our bibliography on wild ruminants of the northern hemisphere.

4. Carnivores

A. Lynx

The lynx parasite study continued to accumulate data on 5 common species of stomach and intestinal worms. A total of 135 "gut strings" was examined. The 5 species of worms (i.e. Cyclospirura sp., 2 Ascaris spp. and 2 Taenia spp.) continue to be abundant in lynx during this period of low lynx numbers in interior Alaska.

B. Canines

Our current work on diseases transmitted to sled dogs or wolves by wild game used as feed is reviewed in the section dealing with caribou. Suffice it to recall that we demonstrated rangiferine brucellosis in sled dogs and presented strong serological evidence of the infection in wolves.

During the past year the alimentary tracts of only three wolves from southeastern Alaska were available for examination. Two of these had light infestations of tapeworms (Taenia spp.). The third animal did not have any gastro-intestinal worms.

5. Marine Mammals

No investigational work was done on marine mammals in 1969.

A paper on several new species of Nasitrema from delphinid porpoises of the eastern Pacific was brought through the "galley proof" stage of publication.

6. Miscellaneous Host Species

Comparatively little work was done on miscellaneous species during 1969.

Of some interest are observations on three yearling muskox which died shortly after they were transplanted from Nunivak Island to the "North Slope" near Barter Island. In one of the two showing clearcut signs of pneumonia, the disease had progressed nearly to the "grey hepatization" stage in most of both lungs. In animals receiving suitable care and in which only a small part of the lung tissue is involved, pneumonic areas may go into a stage of resolution and recovery. In animals in poor condition thrust out into a new and hostile environment, the disease spreads through the lung tissue and death ensues. Suitable veterinary care during and a week or so after game animals are transported to, but not released into new areas would minimize losses of this sort.

Job No. 18.4: Nutritional Physiology of Caribou and Moose

PROCEDURES

The present efforts are being devoted to developing suitable techniques based upon methods which have been applied to domestic animals, and, in one or two instances, to wild ruminants. In general the procedures being used are as described below.

1. Development and testing of a "dry-weight" method of evaluating the fat content of marrow fat depots.

2. Development and testing of a "field-fermentation" method for assessing the quality of herbivore forage in terms of the *in vitro* production of volatile fatty acids and gaseous byproducts.

3. Review of the literature for pertinent information on rumen metabolism and other related aspects of the nutritional physiology of domestic and wild ruminants.

FINDINGS

A. Marrow Fat Studies

In last year's report the "dry weight" technique was only briefly described. Recently it has been written up for publication. Because some of the illustrations and tabular material were inadvertently deleted from or misplaced in the body of the 1968 report I am including the manuscript on the technique in full below.

1. Marrow Fat Estimation

"The Dry-Weight Method: A New Quantitative Technique for Conveniently Estimating Marrow Fat Reserves."

ABSTRACT

Analysis of 34 femur marrow samples from barren-ground caribou reveal that this tissue in this species is a three component system comprised of water, fat and non-fat residue. Both the water and fat, and residue and fat are linearly and inversely related. Because the residue amounts to only 2-6% of the wet weight, the dry weight of the marrow, except at low fat levels, is essentially equal to the weight of fat in the marrow. For maximum accuracy at low fat levels, the dry weight: fat inequality can be corrected by subtracting the average residue value for that fat level from the dry weight. Such corrected dry weights are an adequately accurate measure of femur fat content at all fat levels for studies concerned with marrow fat reserves.

During studies on the diseases and parasites and other biological characteristics of barren ground caribou, Rangifer tarandus granti, marrow fat reserves came under scrutiny. It seemed desirable to employ an objective technique for estimating marrow fat content rather than some visual procedure which under field conditions or with different personnel might be too inaccurate for our purposes.

I decided that conventional extraction procedures were too tedious and more accurate than necessary for routine use on large numbers of marrow samples. It occurred to me that a suitably accurate and quite convenient analytical method could be based upon the inverse relationship which exists in ruminants between body fat and water as reported by Armsby and Moulton (1925) and others according to Blaxter (1962). Assuming that caribou femur marrow fat and water are inversely related, then the only further requirement is that the non-aqueous, non-fat-soluble material (residue) in marrow be either a small or more or less constant proportion of the fresh marrow. This being the case, marrow fat content can then be conveniently estimated with suitable accuracy and precision by simply drying the sample. The dry weight of a sample is the weight of fat in the wet marrow sample. The error in the so-called "dry weight" method can be reduced to a minimum by taking into account the average amount of residual material in marrow samples at various fat concentrations. In order to test the method thirty-four femur marrow samples (17 male, 17 female) were dried and extracted and the aqueous, the fat-soluble and the non-fat-soluble fractions were determined by weighing before and after drying and after extraction.

METHOD

The femurs were allowed to freeze when they were collected and stored intact in a freezer until analysis, usually a few weeks later. Samples for analysis were taken from the central portion of the femur and usually amounted to about 10-30 gms. fresh weight. These were dried in an oven at about 60-65° C. until they were essentially dry (i.e. weights of individual samples varied no more than a few centigrams on succeeding days). The samples were then extracted with equal volumes of chloroform and methanol (160 ml. Total) in a Soxhlet extractor. Almost all of the fat is extracted in the first 2-3 hours and the extraction was normally terminated after 10-12 hours. The non-fat-soluble residue was dried before weighing at 60-65° C. The extract was quantitatively transferred to a beaker and the solvent evaporated off under low heat in a fume hood before weighing. For comparison of the "dry weight," marrow-fat estimation method with the usual subjective, visual estimation method (Cheatum, 1949 and others), samples were visually classified when they were taken for dry-weight analysis. Marrows which were very red and juicy were assigned "visual index" scores of 1. If an unusually poor specimen was encountered after other grade 1 samples had been classified it was scored 1-. Marrows which were hard and creamy white or only slightly pink fringed were assigned values of 4 and those occasional specimens that appeared exceptional were scored 4+. Grade 3 specimens were firm, but uniformly pink to red in color. Grade 2 specimens were pink to red in color and also soft or limber, but not noticeably juicy.

RESULTS

Dry Weight Method of Marrow Fat Estimation

Vital data including femur marrow characteristics of 34 barren-ground caribou collected at Anaktuvuk Pass in April, 1968, are shown in Table 1. The inversely linear relationships of fat to water and fat to residue are clearly demonstrated in Fig. 1. The error in the "dry weight" method due to the non-fatty residue's contribution to the dry weight is shown in Fig. 2 for various dry weight levels. Thus for maximum accuracy in estimating femur marrow fat with the dry weight method, the residue value corresponding to a specific dry weight should be subtracted from that dry weight value to give the corrected percentage of fat. Fig. 3 shows the relationship of fat content to dry weight and "corrected dry weight." Only at very low (> 10%) dry weight values are the discrepancies between the values for dry weight and fat of any relative significance. It is also evident from Fig. 2 that in only 4 of the 34 samples is the absolute error of the correction factor more than $\pm$ 1 %. This correction factor error is only significant at very low fat levels (10% or less) and when considering individual samples. Because it seems doubtful that one can ever demonstrate that marrow fat differences of a few percentage points even at low levels are directly or indirectly significant to the welfare of the animal, there does not seem to be any great need to use tedious extraction procedures to avoid the correction-factor error in the "dry weight" method at low fat levels.

Visual Estimation of Marrow Fat Levels

A comparison of the visual and extraction methods of assessing marrow fat is shown in Fig. 4. Corrected dry weight values for fat could have been substituted for extraction values without significantly affecting the relationship shown in Fig. 4. The amount of overlap between fat values observed for samples allocated to the different visual index grades is small. No doubt a larger series of samples would have shown more overlap. The variability within grades is probably only significant in respect to interpreting the meaning of grade 1 specimens. It seems likely that grade 1 animals with marrow fat values of 24% and 3% respectively may have had significantly different prospects as well as histories. Accordingly even though it seems possible to sort caribou marrow samples by eye into four grades of "fattiness," perhaps even without significant overlap between grades, it does not appear that a four-step visual classification is a sensitive method of comparison, particularly for individual specimens collected now and then.

DISCUSSION

Over the years, procedures employed for estimating marrow fat reserves of ruminants have either involved more or less subjective, visual assessment of "fattiness" or highly objective extraction procedures (Cheatum, loc. cit.; Bischoff, 1954; Ranson, 1965; and others). Visual methods are convenient, but not very accurate or sensitive except at extreme fat levels. Extraction

Table 1. Femur marrow characteristics of caribou taken at Anaktuvuk Pass, April, 1968.[1]

Sex	Age	Visual Index	Water, %	Dry Weight,%	Fat,%	Corrected Dry Weight	Residue, %
F	3-4	1-	88.2	11.8	3.1	2.8	8.7
F	4	1	74.7	25.3	20.5	19.8	4.8
F	6-9	1	90.8	9.2	3.6	3.1	5.6
F	6-9	2	70.8	29.2	24.3	24.1	4.9
F	4	2	46.7	53.3	48.8	49.3	4.5
F	3	2	51.0	49.0	44.5	44.7	4.5
F	6-9	2	47.8	52.2	51.0	48.1	1.2
F	6-9	3	38.4	62.6	58.9	59.0	3.7
F	6-9	3	76.6	23.4	19.5	17.9	3.9
F	5	3	41.7	58.3	54.6	54.5	3.7
F	6-9	3	43.0	57.0	53.7	53.1	3.3
F	4	4	10.5	89.5	87.6	87.3	1.9
F	3	4	23.1	76.9	73.4	74.1	3.5
F	3	4	18.3	81.7	79.3	79.1	2.4
F	3	4	21.8	78.3	76.0	75.5	2.3
F	5	4	27.7	72.3	69.4	69.2	2.9
F	4	4+	21.2	78.9	76.6	76.1	2.3
M	3	1	82.8	17.2	11.5	11.5	5.7
M	4	1	86.1	13.9	7.4	8.0	6.5
M	4	1	87.7	12.3	7.3	6.0	5.0
M	2-3	1	88.3	11.7	5.9	5.7	5.8
M	3	1	71.9	28.1	23.7	22.9	4.4
M	3	1	75.9	24.1	17.4	18.7	6.7
M	4-5	1	80.4	19.6	13.7	13.9	5.9
M	3	1	87.8	12.2	5.6	6.3	6.6
M	4	1	88.4	11.6	6.4	5.5	5.2
M	6-9	2	59.0	41.0	36.8	36.3	4.2
M	6-9	2	52.5	47.6	45.6	43.3	2.0
M	2	2	51.2	48.8	44.3	44.6	4.5
M	2	2	66.0	34.0	28.8	29.1	5.2
M	2	2	47.1	52.9	49.7	48.8	3.2
M	2	3	22.8	77.2	74.4	74.4	2.8
M	1	3	42.2	57.8	53.8	53.9	4.0
M	4	3	42.2	57.8	54.9	53.9	2.9

[1] Fat determined by chloroform-methanol extraction. "Corrected dry weight" calculated using specific correction factors obtained from Fig. 2.

procedures are theoretically as potentially accurate as desired, but are too time consuming for large scale use, except perhaps under very unusual circumstances. Recently Greer (1968) described a so-called "compression" method for estimating femur marrow fat reserves in Wapiti and other wild ruminants. While this procedure is comparatively quick and easy to use, it can only be described as semi-quantitative and may not really be more accurate than a visual procedure used by an experienced person. This may be particularly true when dealing with a few marrow samples now and then, rather than a large series at one time.

To the best of my knowledge, no one has previously reported using what I have described above as the "dry weight" method of measuring marrow fat. Bischoff (loc. cit.) propounded a correllary of the "dry-weight theory" when he stated in a report on California deer, "A high fat content is found in all samples when expressed on the dryweight basis." This suggests that in Odocoileus spp. as in Rangifer the marrow is essentially a three component system comprised of water, fat, and residue, this last component only amounting to a small fraction of the fresh weight. Whether this is also true of other ruminants is not now known. However we are now examining marrows from moose, sheep and other Alaskan big-game ruminants to determine the applicability of the dry-weight method to these species.

It seems that the "dry-weight" method has the advantage of being as accurate, or nearly so, as the extraction method yet is substantially quicker and less expensive to use. All that is needed is a "$40.00 centigram" balance and some kind of simple drying device. For those inclined to use highly combustible diethyl ether or "petroleum ether" as part of the solvent system in an extraction procedure, the dry-weight method also offers increased safety from potentially substantial laboratory accidents.

The "dry-weight" method is slower than the "visual" or "compression" methods, but with experience it can be effectively speeded up. For example, if the variation in tare weights of the drying containers amounts to no more than $\pm$ 1% or so of the dry weight of the samples one can simply use an average tare weight and not bother to tediously weigh each to the nearest centigram. Use of plastic vials and suitably heavy samples of marrow allow this simplification without detriment to the procedure. Under normal circumstances it will be found that dry weight is obtained with only small error within a certain time. At the end of a "normal" drying period 2 or 3 samples can be weighed and then reweighed the following day. Although the "dry-weight" method may extend over several days to completion, the investment of time per sample is small when batches, rather than a single or a few samples, are run through the procedure.

Assuming that femur marrows of other ruminants are qualitatively and quantitatively similar to that of Rangifer, there seems to be little justification for using any other procedure than the "dry-weight" method for estimating marrow fat reserves. This method is nearly as convenient as the "visual" and "compression" procedures and for practical purposes as accurate as various extraction procedures. It is inexpensive and employs sturdy equipment. What more can one ask?

Figure 1. The relationship between water, fat and non-fat residue in caribou femur marrows.

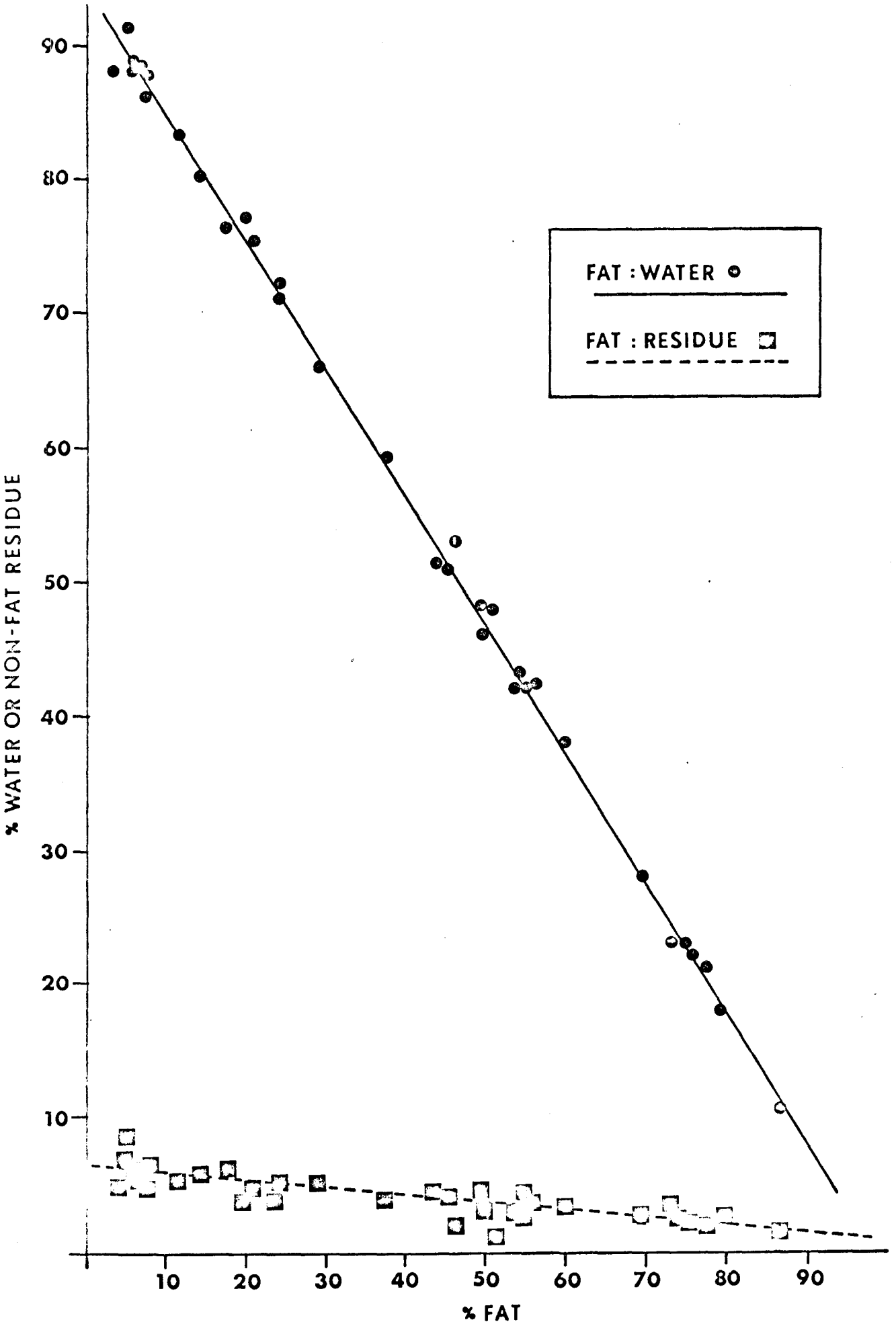

FAT : WATER
FAT : RESIDUE
% WATER OR NON-FAT RESIDUE
% FAT
10
20
30
40
50
60
70
80
90

Figure 2. Variation of non-fat residue with dry weight.

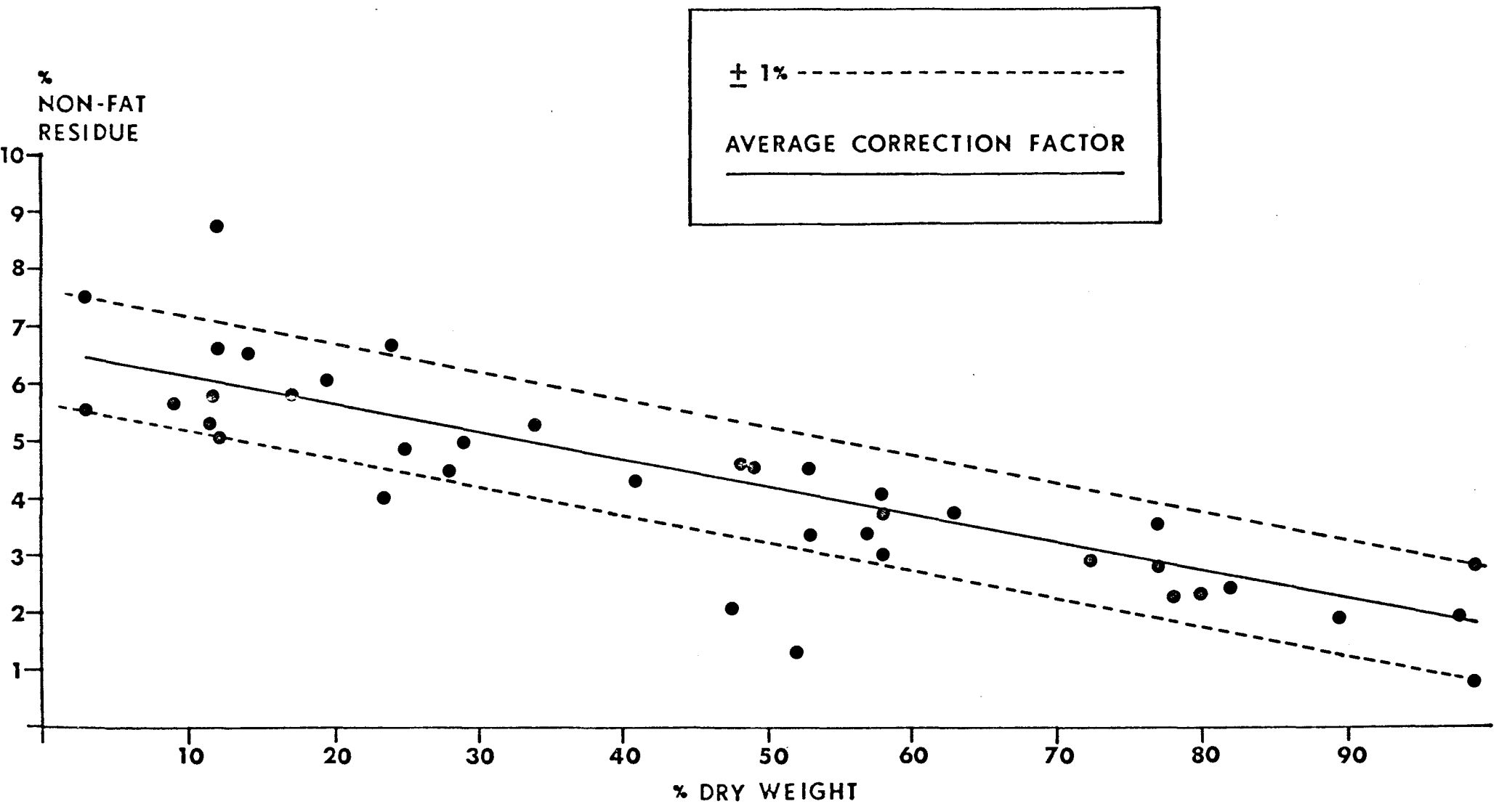

± 1%
AVERAGE CORRECTION FACTOR
%
NON-FAT
RESIDUE
10
9
8
7
6
5
4
3
2
1
10
20
30
40
50
60
70
80
90
% DRY WEIGHT

Figure 3. Comparison of dry weight, "corrected dry weight" and fat content of femur marrows, dry weight corrections determined from Figure 2.

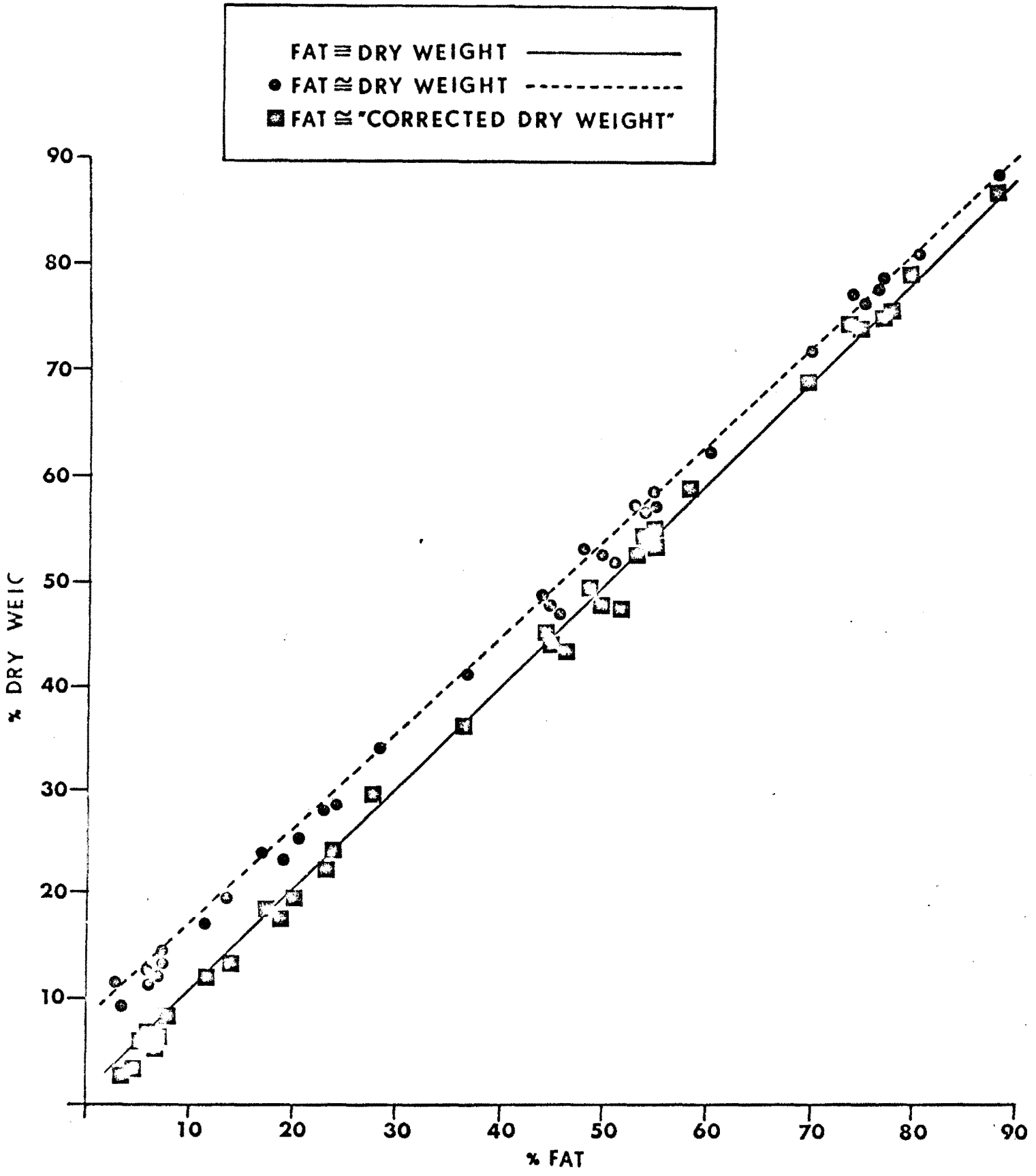
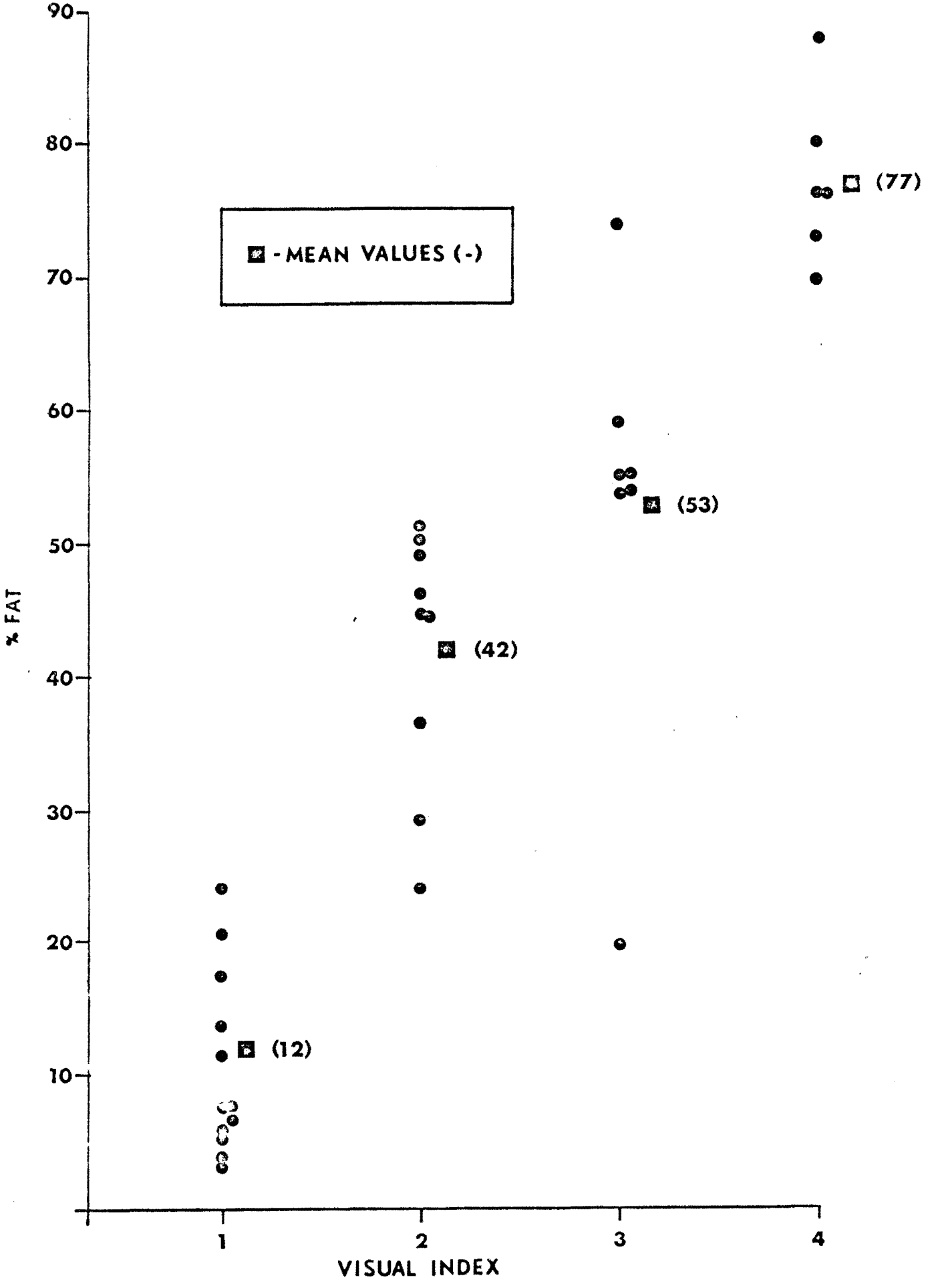

FAT ≡ DRY WEIGHT
FAT ≅ DRY WEIGHT
FAT ≅ "CORRECTED DRY WEIGHT"
% DRY WEIC
% FAT
10
20
30
40
50
60
70
80
90

Figure 4. Comparison between extraction and visual assessment of femur marrow fat content.


90
80
70
60
50
40
30
20
10
% FAT
■ - MEAN VALUES (-)
(77)
(53)
(42)
(12)
1
2
3
4
VISUAL INDEX

2. The Relationship of Marrow Fat to Other Indicators of Conditions

To the best of my knowledge no one has yet investigated the significance of marrow fat stores as an indicator of condition in barren-ground caribou, or any other species of Rangifer. Observations on deer reported by Cheatum (1949), Bischoff (1954) and Ransom (1965) indicate that marrow fat levels are useful, but not infallible indicators of condition in deer. Bischoff (loc. cit.) reported that 34 of 36 deer having no more than 39% marrow fat levels were in "poor" or "very poor" condition (i.e. other fat depots depleted or nearly so). The other 2 animals of the 36 were rated as in only "fair" condition. On the other hand, 31 other animals also rated as in "poor" or "very poor" condition had marrow fat levels of 80% or more. Accordingly, while one evidently can safely conclude that black-tailed deer with low marrow fat (i.e. less than 40%) have significantly depleted fat reserves overall, high marrow fat levels are also sometimes found in deer with depleted overall fat reserves. Since it generally seems to be the case that marrow fat is mobilized to any degree last of all, it is easy to see why deer can be found in which the marrow depots are still "full" or nearly so while the non-marrow depots are "empty." Ransom (loc. cit.) reported that in white-tailed deer the marrow fat did not begin to significantly decline until kidney fat was essentially exhausted. Thus for deer one can conclude that low marrow fat levels are most often of significance while high levels are frequently not.

Another method of measuring condition has been applied to black-tailed deer by Bandy et al. (1956). Working with experimental animals they have demonstrated that the "actual condition" (i.e. weight) of an animal is correlated with the "heart girth" while the "optimum condition" (i.e. weight) of an animal on a high plane diet is correlated with hind foot length, i.e. skeletal size. Thus the ratio of condition estimated from heart girth over condition estimated from hind foot length will be 1.0 or larger in animals in good condition or less than 1.0 in animals in poorer condition. The ratio, $\frac{\text{condition (heart girth)}}{\text{condition (hind foot length)}}$ was termed the index of condition.

Because heart girth, hind foot length, total and dressed weights and other body measurements are comparatively easy to make with satisfactory accuracy and precision, while measurements of non-marrow fat depots are difficult, it was decided that we would first examine the relationship of femur marrow fat levels to index of condition (I. C.) in caribou.

The relationships of marrow fat levels to several "condition indexes" based upon body measurements are shown in Figures 2-5. Although more data are needed on animals showing lower marrow fat levels (i.e. less than 50%), it appears that overall condition as measured by so-called "condition indexes" only shows correlation with marrow fat at low marrow fat levels. This is consistent with observations on black-tailed and white-tailed deer by several authors which were discussed above. It appears that in caribou as with other species the marrow fat is not extensively mobilized until other fat depots are essentially exhausted or nearly so. Thus high marrow fat levels may be found in individuals either at the peak of condition or in others in only fair condition in which full-scale mobilization of marrow fat has just or is about to begin. The data on black-tailed deer and that thus far available

FIGURE 2

Variation of Femur Marrow Fat Content Compared with the Girth/Hind Foot Length "Condition Index", Spring 1968 and 1969, Adult Females

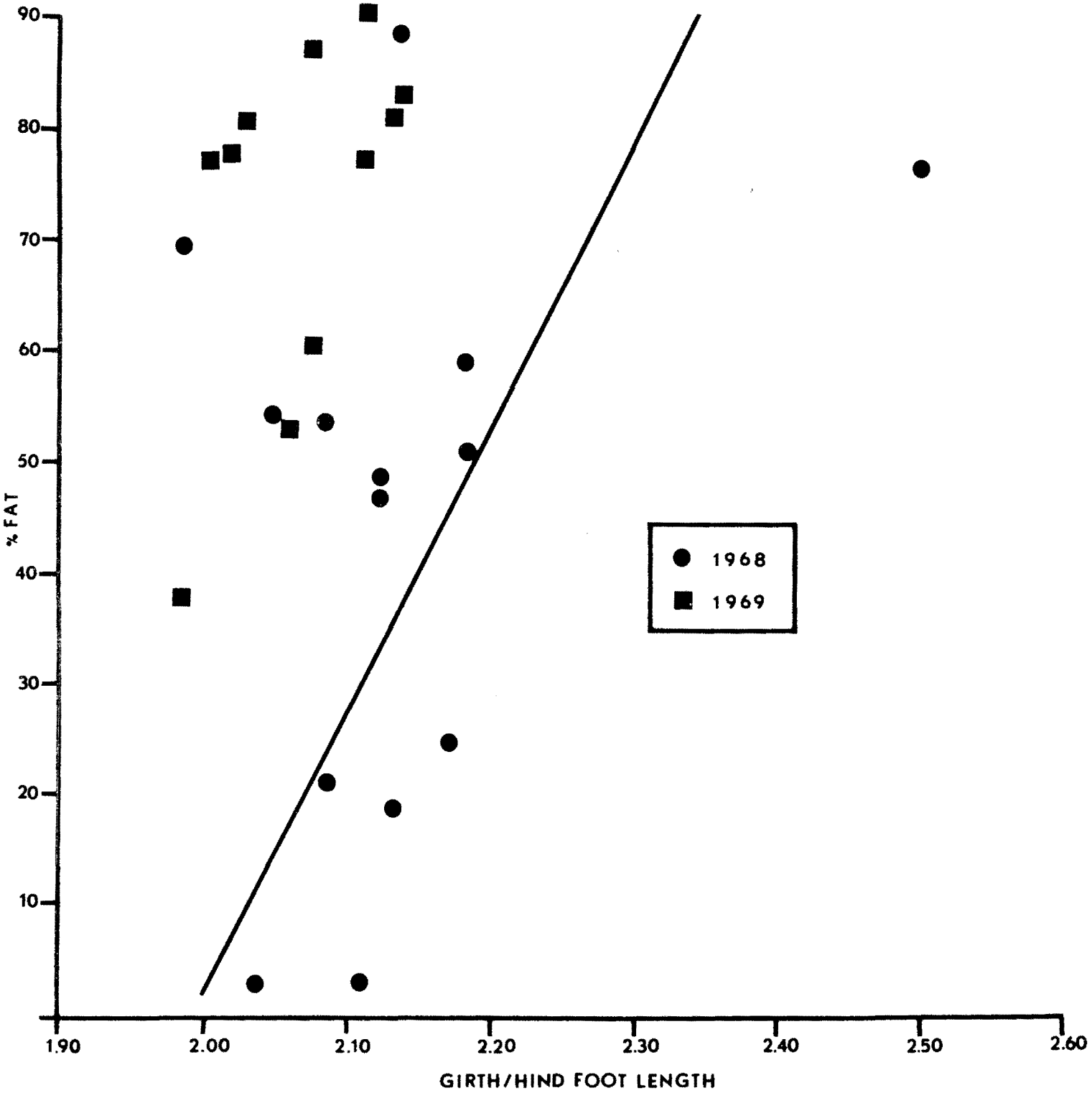
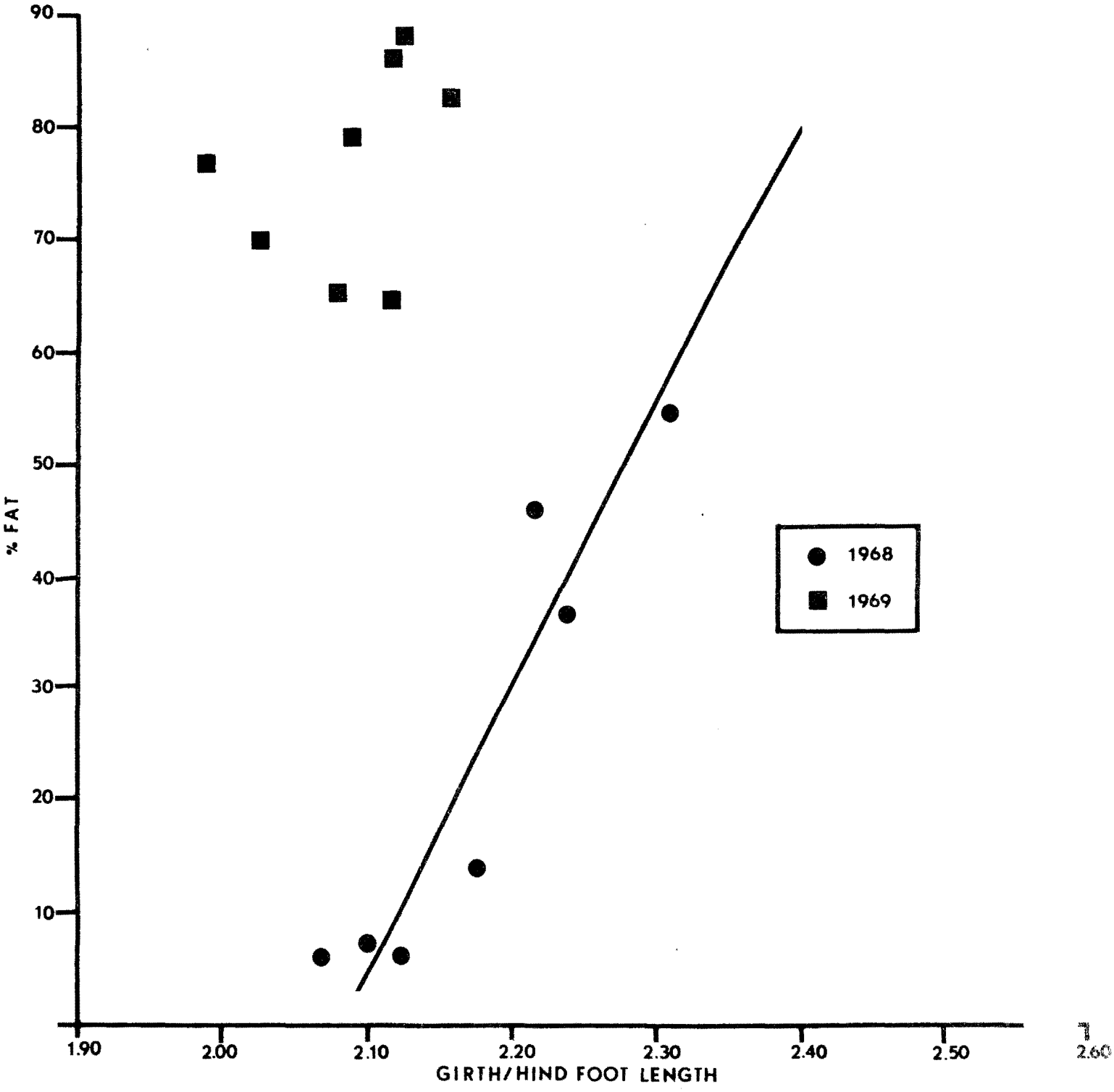


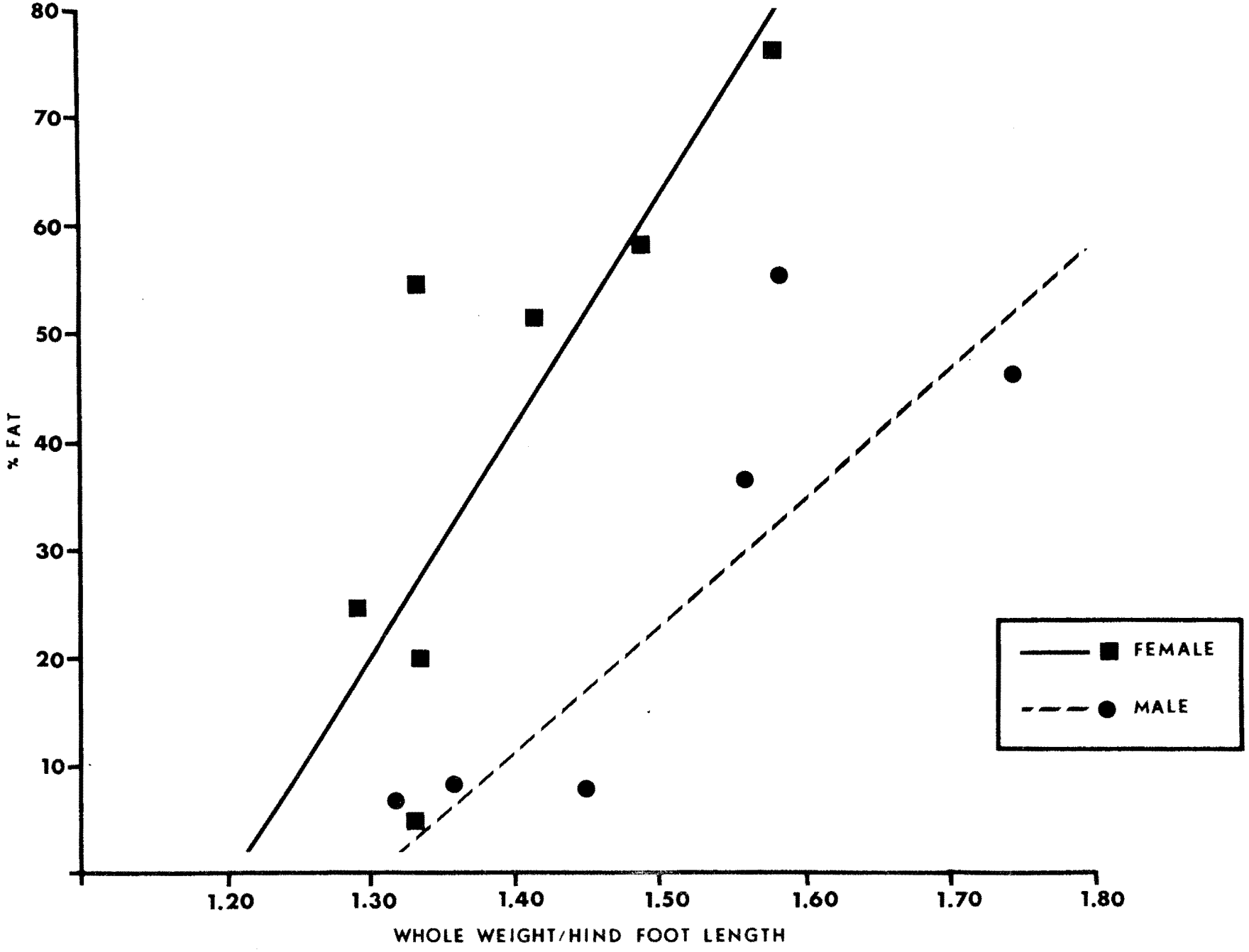
FIGURE 3

Variation of Femur Marrow Fat Content Compared with the Girth/Hind Foot Length "Condition Index", Spring 1968 and 1969, Adult Males



FIGUR[illegible] 4

Variation of Femur Marrow Fa[illegible] ontent Compared with the Whole Weight/Hind Foot Length "Condition Index", Spring 1968



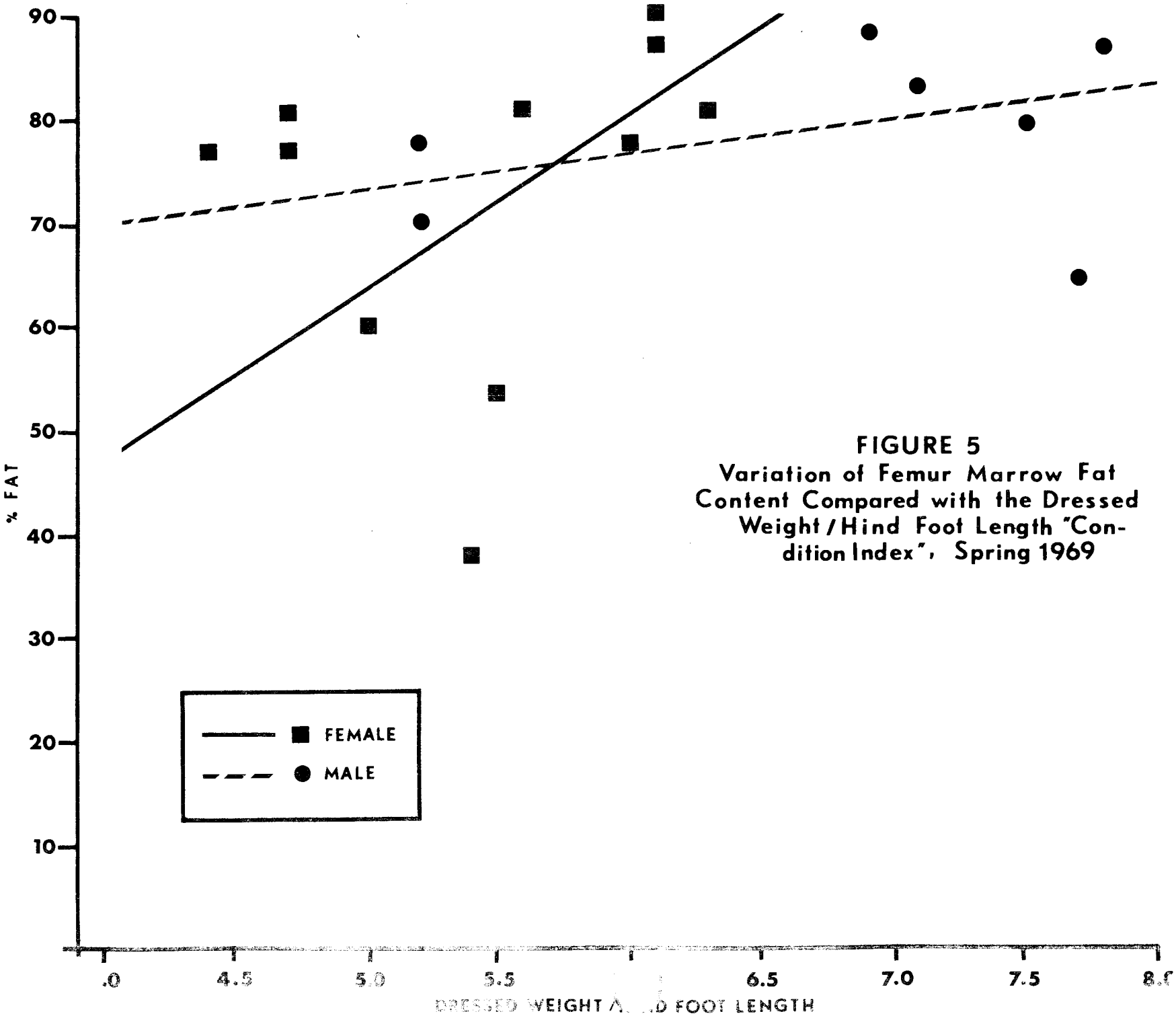


FIGURE 5
Variation of Femur Marrow Fat Content Compared with the Dressed Weight / Hind Foot Length "Condition Index", Spring 1969

FIGURE 6
Hypothetical Visualization of Mobilization of Fat From Marrow and Other Depots

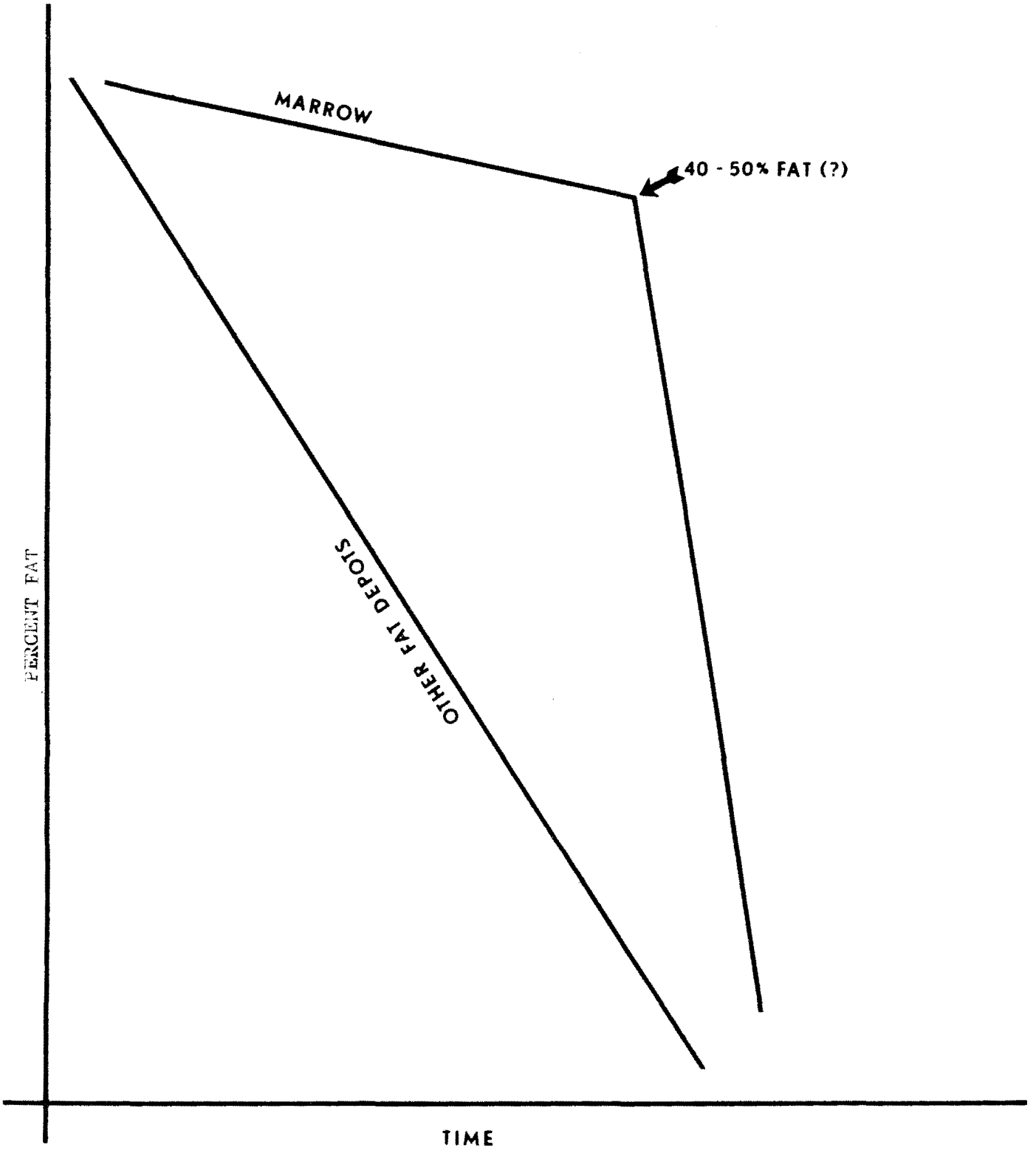
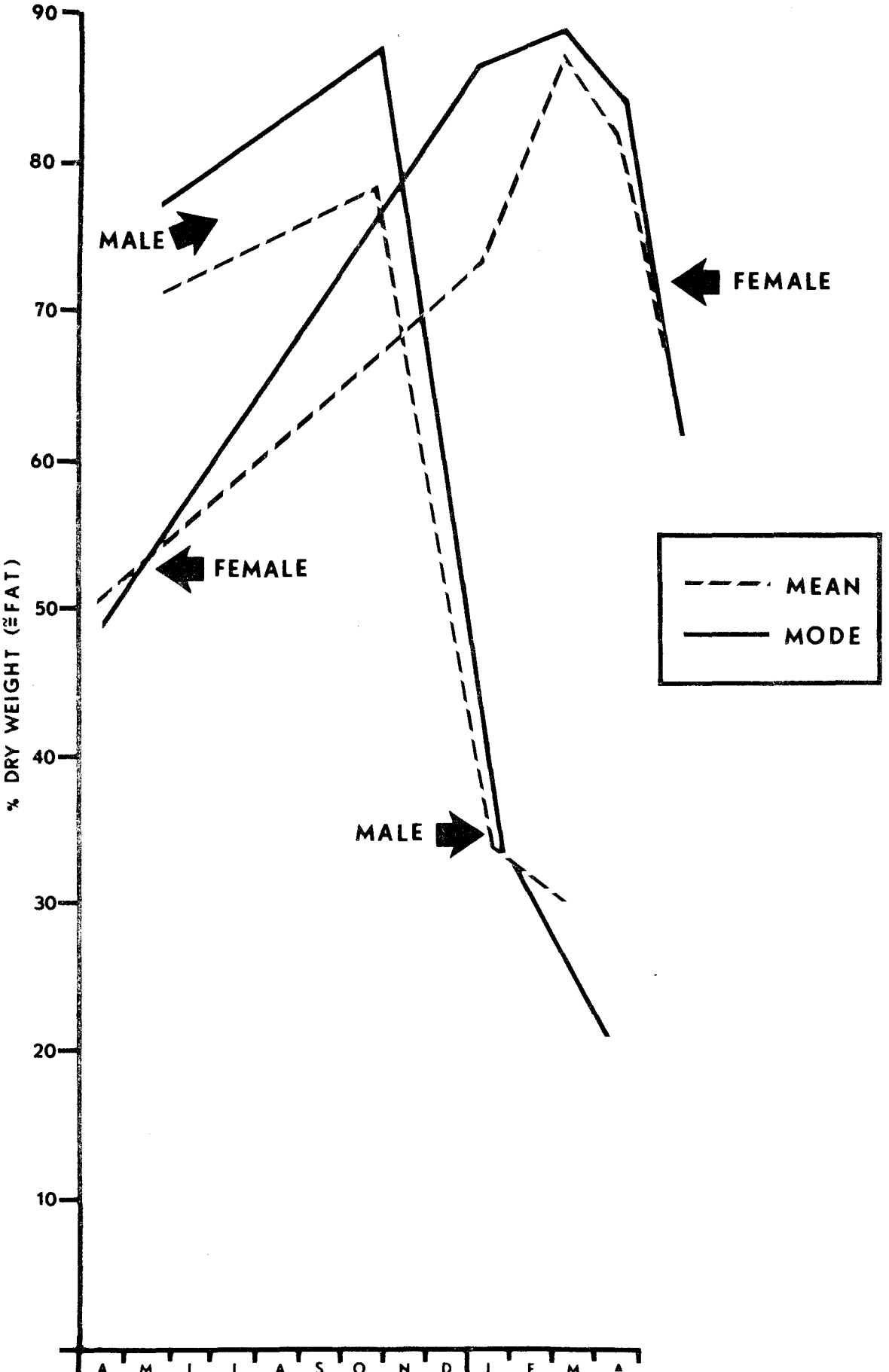


FIGURE 7

Seasonal Variation of Femur Marrow Fat in Caribou of the Arctic Herd, 1967-1968



on caribou suggest that marrow fat levels of about 40-50% are indicative of the onset of full-scale mobilization of marrow fat. Considerably more data are required before we can identify this critical marrow fat value. It is my speculation at this time that if one could measure the rate of mobilization of marrow fat continuously from high to low fat levels one would find a point at which a substantial change in the rate occurs. This inflection would coincide with the exhaustion or severe depletion of other more readily mobilized fat depots and would likely occur when marrow fat had fallen to 40-50%. This hypothesis is visualized in Fig. 6. We expect to gather additional data bearing on this hypothesis during the coming field season.

3. Seasonal Variation of Marrow Fat Levels

During the fall, winter, and spring of 1967-68 it was possible to obtain a relatively large number of femurs for marrow fat analysis from animals taken by subsistence hunters at Anaktuvuk Pass. These data are summarized in Fig. 7. Perhaps the most striking feature of the data is the apparent great disparity between male marrow fat levels in the springs of 1967 and 1968. The data suggest that male marrow fat levels "peak out" before the rut and then may not recover during the ensuing winter after the rut if conditions are not favorable. On the other hand, female marrow fat apparently did not (does not?) reach maximum levels until several months later in the winter and then subsequently declined a good deal less than male marrow fat. The delayed "peaking" of female marrow fat may be related to the demands of lactation and other activities associated with calf rearing during the growing season. Whether marrow fat levels of bulls are good indicators of environment stress and/or quality of winter range remains to be seen.

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