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AD HOC SUBCOMMITTEE ON LEISHMANIASIS

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Cutaneous leishmaniasis and its effect on the military is discussed. This disease has long been known to be endemic to the Panama Canal Zone, and similarly, the disease is endemic in Brazil and in other Central and South American countries. Outlines of studies of leishmaniasis at Ft. Bragg, Immunofluorescent antibody studies, culture techniques in laboratory diagnoses, and therapuetic studies on leishmaniasis using Pentostam are included.

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ARMED FORCES EPIDEMIOLOGICAL BOARD

AD HOC SUBCOMMITTEE ON LEISHMANIASIS

8E069 Forrestal Building
Washington, D.C.

April 11, 1978

C O N T E N T S

P R O C E E D I N G S

LTC ERICKSON: This is a prime example of Murphy's law. Even the projector that was in the booth was not there, and it is out. Anyway, we will start off. First of all, on behalf of the Armed Forces and the Surgeon General, I want to thank the AFEB member and consultants who have come this morning.

We fully appreciate that you go through a good bit of personal sacrifice and considerable inconvenience to come to these meetings, and we do appreciate it.

The meeting will be recorded in order to give us the detailed minutes that are required by the Federal Advisory Committee Act and in order to have as useful a transcript as possible, if you will state your name before you speak or unless the Chairman, Dr.Benenson recognizes you, please state your name,and then that will be on the record. There are telephones and restrooms down the hall to the left. At the coffee break if you go down to the fifth floor and across the walkway between the two buildings over on the B Corridor there is a snack bar. For lunch, if we run into the afternoon, we will go down to the first floor and across the street, and there is a cafeteria over there in the other section, of course.

I think that will take care of it. The consultants who are here have an expense sheet before you, and if you

will check with Mrs. Eldridge, she will give you the information you need to fill those out for the expenses. I hope to get a projector here in a few minutes, and until then we will just have to go without it. I am sorry.

Dr. Benenson?

DR. BENENSON: I think the first thing we should do is for the sake of the record to identify ourselves and for the sake of the very interested audience that we have here. I had personally not realized how much interest there is in leishmaniasis in the US. When you are in Panama, there is a lot of interest in leishmaniasis. It is encouraging to see that other people, too, are interested in such problems.

I gather we don't have loudspeaker systems, so having tested the audibility of the room, I will request now that all discussions be carried at a volume audible throughout the room.

Now, by way of introduction, I am Dr. Beneson. I am Director of the Gorgas Memorial Laboratory in Panama.

(Introductions.)

DR. BENENSON: I am sorry, there are too many behind us to get all the individual introductions. Our problem, obviously, has to do with leishmaniasis, and Dr. Cutting will give us the details of the problem, please?

LTC. CUTTING: Thank you, Dr. Beneson.

The problem that we are faced with today, as you all

know is cutaneous leishmaniasis and its effect on the military. The Panama Canal Zone has long been known to be endemic for cutaneous leishmaniasis, and similarly the disease is endemic in Brazil and in other places in Central and South America.

US military personnel are permanently stationed in certain of these areas, particularly in the Canal Zone. In addition, we have, at times, large numbers of troops undergoing training, particularly in jungle operations that come down from the United States to the Canal Zone, and we have small numbers that participate in training in Brazil.

Cases of cutaneous leishmaniasis occur in these personnel, both in those permanently stationed in the areas, as well as in trainees. The group which has brought it to our particular attention now is in the trainees and in recognition of this there has been a prospective study initiated in personnel that go down from Ft. Bragg to the Canal Zone for training.

Major Takafuji is going to talk about this study and some of its early findings, at least, in a few moments. For the moment though, let me just say that we have, indeed, identified cases of leishmaniasis in this group, and there have been a number of other questions that have been raised as a result of this study and of other people talking about problems, and we submitted a memo to the Board which I believe everybody at the table, at least, has which mentions some of

these questions. What we are seeking advice on is particularly upon therapy, upon medical follow-up, screening techniques and the blood donor program.

As far as therapy goes we are currently using or at least had been up until recently, the CDC protocol, using Pentostam. There has been a new protocol developed by the people at Walter Reed using the same total dose but different dosage schedules. There have, also, been questions raised about whether or not other drugs might be worthwhile to investigate.

In terms of medical follow-up, this is a very sensitive subject today, although we haven't talked about cutaneous leishmaniasis before in relation to follow-up. The question which we think about is how long should people be followed? What types of procedures should be employed in following them? Who should be followed and so on?

Related to this are screening tests. Who should be screened? What types of procedures should be used, culturing, skin smears, skin testing, the Montenegro test? What are the advantages and disadvantages of each of these? And a fourth area, the blood donor program, we would like the opinion and advice of the Subcommittee and the Board on what, if any, risk exists for those who are infected with leishmaniasis in terms of transmission via blood donations. What restrictions, if any, should be imposed upon those who are infected, on

those who are exposed without clinical disease or on any other group which we may be able to identify?

I should note now that we have notified the major commands that are involved with the training exercises, particularly the training exercises as to the possibility of cutaneous leishmaniasis in those personnel going to Panama and returning. We have alerted them to current methods of therapy and treatment with advice on what procedures to follow. There is work underway to evaluate repellents, both old and new, but I guess my last question to the Board is are there other areas that we should be investigating as well, and if so, could we have your recommendations to that effect?

DR.BENENSON: Thank you. You have enunciated the questions that are in the program, that we will address this afternoon. Will you remind us of the subsidiary questions at that time and make sure that we have addressed them?

For the sake of clarifying the total picture, cutaneous leishmaniasis, as you have outlined it so far is a disease that troops acquire in Panama, but I think it is worth, for the record and for global thinking, to record where else US troops not currently deployed might encounter such problems.

How about filling that out?

DR. WALTON: Yes, this is a rather large order without getting into details, but I think the current practice

is to use the plural when speaking of leishmaniasis, to speak of leishmaniases because there are a variety of parasites with widely differing clinical manifestations of the disease. Leishmania tropica or so-called "oriental sore, Delhi boil," various local names, has a very wide distribution through the Mideast, Africa. The visceral forms are, also, widely distributed throughout most of the Orient and surprisingly enough it is a real entity in the Americas also, although you won't find this in current texts. This is something that is just coming out. So, I would say that it is practically a worldwide problem in the subtropics and tropical zones.

DR. BENENSON: Dr. Farah?

DR. FARAH: My particular experience is in tropical Leishmaniasis, and I think we are beginning to see, at least in areas that are now being developed and opened up for habitation and industrialization and oil and all that and even new endemic areas of leishmaniasis. For instance, around the Mediterranean Basin, in Libya, in Afghanistan, in Pakistan, certainly there are very serious problems of leishmaniasis. In Iran --

DR. BENENSON: Cutaneous?

DR. FARAH: Cutaneous leishmaniasis, yes. Of course, there is also the other form of leishmaniasis which Dr. Walton referred to, and that is the visceral leishmaniasis. It is interesting to note that at least our experience is

that the incidence of one is almost inversely proportional to the incidence of the other. So, in areas where there is a high incidence of cutaneous leishmaniasis, we see less systemic leishmaniasis and vice versa. There is now a very serious epidemic of systemic leishmaniasis in England.

There is very recently a report of both cutaneous leishmaniasis and also visceral leishmaniasis imported from Northern Italy, so that I suppose the area of the Mediterranean is a well-known and documented focus for both types of leishmaniasis.

DR.BENENSON: You say, "Northern Italy"?

DR. FARAH: Well, it has been known previously in the southern parts of Italy but the recent report is from the North.

DR.BENENSON: Dr. Marsden, any additions?

DR. MARSDEN: Yes. As regards the situation, perhaps I should comment more on South America, since that is where I have known it, although I could speak on Africa and the Mediterranean.

The situation in Brazil which is half South America is that in every state that it has been looked for in the Brazilian states it has been found. Really, reports have not appeared when an investigation has been mounted. Quite a high incidence has been found based on a hospital study. An example is Goias in the center of Brazil which is the

state in which the Federal District, where Brasilia was constructed exists and William Barbosevelt, five years ago, he reported 110 cases seen in two years at his clinic in Goiago(?) and this was a state which if you look in Samuel Couseau(?) which is the epidemiological reference really for these things in Brazil, there is no note in Samuel Couseau's classical text of the disease existing in Goias, and so it is quite likely, actually, it exists throughout the states of Brazil, in all the areas.

So, in the north of Brazil we know that cutaneous mucocutaneous leishmaniasis exists, in the Guyanas, in Venezuela as you pass up towards the Central American stem. To the south, the incidence appears to be low in Argentina, just in the north. Peru has its own classical form, of course. Chile, I am told, and I have been to Chile, that it is rare.

So, really the involvement in South American with this form which was initially characterized by Gashbar Veaneris(?), the incidence is notable.

The situation as regards kala-azar, visceral leishmaniasis in South America is that it occurs in Brazil in various coastal states and also occurs in Venezuela, and there are reports from other parts of the Americas.

There has been an attempt to raise a new species, a subspecies of Leishmania donovani, Leishmania donovani shagasi(?) based on the characteristics of the type of

visceral leishmaniasis, because as we know in visceral leishmaniasis the behavior clinically and particularly the chemotherapeutic response is extremely variable depending on which type of visceral leishmaniasis you are talking about.

My worse cases have been those from the Mediterranean, the only cases in which I have seen splenectomy employed as a way of diminishing the load of parasites after repeated chemotherapy with pentavalent antimonial pentaminate(?)

Fortunately the situation in Brazil as regards to leishmaniasis chemotherapy is good. It responds almost as well as Indian kaka-azar, so that I have never seen a case, and Protta has not either who has more experience than I have and wrote a monograph on it, we have never seen a case where it has resisted pentavalent antimonial, a case of visceral leishmaniasis, I stress.

DR.BENENSON: What about Africa, other than the Mediterranean Basin?

DR. MARSDEN: Well, of course, there is a tremendous amount of leishmaniasis extending down the the East into the Rift Valley. The Sudanese form of leishmaniasis which is unusual in that they have both visceral and cutaneous forms, and sometimes the visceral form has cutaneous manifestations. Kala-azar is endemic in the Rift Valley in Kenya, and it is, I would say, the second worse form of leishmaniasis to treat after Mediterranean. I am talking

about visceral leishmaniasis, but in terms of kala-azar the worst type of kala-azar to treat is the Mediterranean. The second worst is in the Rift Valley and the Sudan, and Indian responds well. The Chinese, I have no experience with that, but the literature says that that responds well, and the Brazilian responds well.

A very interesting thing about the Brazilian is where did it come from. I don't want to dwell on this, but -- do you want me to dwell on that?

DR. BENENSON: No, not yet. Let us not dwell on it yet. We do have a program. We are running a little behind, but I want to develop the picture as we go. We will let that go for the time being.

Any other comments on the geographic distribution beyond what we have? All right, Brice?

DR. WALTON: Just one. The United States has now joined the ranks of the elite group of countries having it, since we now have at least three, I have talked on this, cases of cutaneous leishmaniasis acquired inside the United States. Is that right, Mike? Have there been more since then?

DR. SCHULTZ: I knew of two. Our staff investigated two. I am not sure where the third is.

DR. BENENSON: Specifically from where?

DR. SCHULTZ: Texas.

DR. BENENSON: Is your third one a Texas report?

DR. WALTON: It is presumed the one before that was from the same area or possibly Tomalipas(?) Mexico, which is also not a known endemic area. Remember the case published just a few years before that.

MAJ. HENDRICKS: The literature recently has reports from South Africa as well. I have some isolates from that. It is cutaneous form in South Africa.

CAPT. GUNNING: Captain Gunning from Ft. Pendleton. While I was in Taiwan they were researching it, and we uncovered two cases of cutaneous leishmaniasis on the Island of Taiwan where the disease has never before been reported.

Combing the Japanese literature we were unable to discover anything. The only thing we have ever seen in Taiwan were imported cases of kala-azar from the China mainland when Chiang Kai-sheck's troops specifically came over. They brought with them several people who were infected, and the other source of disease on the island was the leprous area where an occasional case of post-kala-azar dermoleishmanpid had been secreted away as a leper, and beyond maybe a dozen or so reported cases from that ingress of the Chinese Nationalist troops, no other cases of visceral leishmaniasis were known on that island, and then the two cutaneous cases arose in the very same village in the Central Highlands.

We were, also, unable to find infected sandflies in

that village. It must be a very low density focus.

DR. BENENSON: Dr. Farah?

DR. FARAH: I don't know whether you want to also mention the other from of cutaneous leishmaniasis, the so-called "diffuse cutaneous leishmaniasis" which exists certainly in South America where it has been described and also, in Ethiopia which may be an interesting area these days.

DR. BENENSON: I think I have achieved my purpose, and that was to not let Colonel Cutting get away with the idea that it is a disease restricted to the country where I live. It is a worldwide problem, and from the military perspective, I think it has to be considered as a worldwide problem and not just a problem of training troops that are being sent to Panama for a, what is it, three-week, six-week exercise? How long is the exercise?

LTC. CUTTING: Three weeks.

DR. BENENSON: A three-week exercise. All right.

If I remember right, our next was going to be a more precise set of information by Dr. Takafuji on what has gone on among the people who have come back from Panama.

We will talk about Panama now.

MAJ. TAKAJUJI: As mentioned earlier, cutaneous leishmaniasis has indeed been reported in military personnel and civilian travelers visiting tropical and subtropical

areas of the world for several years now. The majority of the most recently reported cases, however, of New World cutaneous leishmaniasis have been in US servicemen assigned to Panama for varying lengths of time. Many of these cases were participants in the jungle warfare training course.

This morning what I shall do is present a summarization of the incidence of this disease in Ft. Bragg personnel, first of all, within the last two years and then describe a surveillance study that is presently in progress with a typical unit that had been deployed to the Canal Zone last year.

The number of cases seen at Ft. Bragg in 1977, is a definite increase over what has been reported previously. Between December 1976 and January 1977, two enlisted personnel from the 82nd Airborne Division were diagnosed as having cutaneous leishmaniasis.

In the summer of 1977, an additional five cases were diagnosed. All of these individuals had been to Panama for training two to six months prior to the time of diagnosis and all gave a history of having had sores on return from Panama.

If I may see the first slide, please?

I would like to present just two examples of the cases that we saw last year. This individual is a 25-year-old black male who developed a primary lesion on his left wrist

while in Panama.

Approximately three months after his return to Ft. Bragg he developed secondary lesions on his elbow, as well as lymphadenopathy extending up his arm.

If I may see the second slide, please?

This is the second lesion on his elbow. The second case that I would like to present is a 37-year-old male, Special Forces individual.

Third slide, please?

He developed this crusted ulceration on his elbow that began as a small crusted papule.

Slides off, please?

What I am going to pass around to the members of the Board is a folder that includes some pictures of these cases so that you can look at them a little closer, as well as another individual who was seen, one of the individuals who was seen in January of this year at Ft. Bragg, and those pictures reflect the scars.

Also, in that folder are some pictures of the unit that I will be discussing that went through jungle warfare training school in November of last year.

In 1977, four individuals from Special Forces Units at Ft. Bragg were sent to Brazil for several months of training in jungle warfare tactics. All four of these individuals developed ulcerations that were consistent with

cutaneous leishmaniasis. In at least three of the four individuals organisms have been recovered from ulcerative sites.

At least one individual has not responded to several courses of Penostam, as well as Amphotericin B, with continuing positive cultures from his initial lesion site.

The ten culture-proven cases that I have described in 1977 appear indeed to indicate an increasing incidence of clinically apparent illness, but it is recognized that increased awareness with raised clinical suspicion may also be playing a role here.

Probably one of the reasons why more cases are being noticed lately is related to the development of the unit training concept in Panama where battalion-sized units from the same posts are now being trained as one unit. Hence, disease in a unit of several hundred persons becomes more apparent when this unit returns to its original post.

In November 1977, over 650 military personnel from the First Battalion 325th Infantry belonging to the 82nd Airborne Division were deployed to Ft. Sherman in the Canal Zone for jungle warfare training.

If I may see the next slide, please?

The area that we are concerned about, of course, is the Canal Zone and in particular this part of the Canal Zone with Ft. Sherman sitting on the peninsula here.

If I may have the second slide, please?

This is a blowup of the Canal Zone. Just to orient you a little bit, north is this way. Ft. Sherman is located here on this peninsula, but the training area that we are talking about extends in general across half of the Isthmus of Panama in this area here. Training takes place in this area.

The 624 participants then underwent two and one-half weeks of intensive training following an initial drop in the Gatun drop zone. The Gatun drop zone is located here.

The 624 participants then underwent various training covering areas such as jungle skills, squad and company combat tactics, navigation, water-borne operations, propelling from helicopters, as well as patrolling.

If I may see the next slide, please?

Some of the publicity that was generated down at Ft. Bragg reflects the type of training that was being conducted, and if you could refer to the photographs in the book that I have passed around, I think you will get a general idea of what exactly happened.

Slide off, please?

The training pattern for this particular battalion was very similar to that of previous groups from which cases of leishmaniasis were identified earlier in the year, and it culminated with a field exercise that involved the entire

battalion during the last week of the exercise.

The group returned to Ft. Bragg on December 10, after initial deployment on November 19.

A prospective surveillance study of this battalion was conducted by the Health and Environment activity at Ft. Bragg and the Division Surgeon, 82nd Airborne Division, with the support of the Division of Preventive Medicine, Division of Communicable Disease and Immunology and the Division of Medicinal Chemistry at the Walter Reed Army Institute of Research.

The primary objectives of this study were to first of all determine the morbidity from leishmaniasis by determining the clinical and subclinical attack rates based on clinically apparent signs and symptoms, as well as serologic data; number two, to correlate the incidence of disease with demographic information of the participants and also currently available information on the distribution of sandfly populations in the area in an attempt to identify possible risk factors; number three, to identify individuals in the group with evidence of infection, thereby allowing line commanders to be aware of the possible need to replace these individuals in situations of realistic deployment, and finally, number four, to determine the expenditure of time, financial resources and personnel required to conduct surveillance for cutaneous leishmaniasis, realizing that

the need for the development of the most efficient means to conduct continuing surveillance was in order.

Demographic data, including age, rank, race, years of active duty and previous exposure to areas of the world endemic for leishmaniasis were collected.

In November of last year pre-deployment sera were collected from all of the 624 participants one week prior to departure from Ft. Bragg.

In January of this year approximately four to six weeks following return of the unit to Ft. Bragg, post-deployment sera were collected from 612 of the 624 individuals or 98 percent of the participants.

Dermatologic evaluation of all of the participants was, also, performed by a trained physician at the time of the second bleed. Individuals were, also, questioned about the occurrence of skin problems or fever during or immediately following the training exercise through the use of a questionnaire form.

Individuals with suspicious lesions were cultured using Schneider's insect media. Techniques will be described by Major Hendricks later on this morning. Paired sera were then tested for the presence of antibody utilizing the indirect fluorescent antibody test which will be discussed after my presentation by Colonel Diggs.

Results, also, will be presented at that time.

During the last week of this month, depending on the availability of the members of the unit and commitments to continuing training exercises, a third blood specimen will now be collected from all participants who are still present at Ft. Bragg.

The reason for the third blood specimen is to detect, hopefully, any late serologic converters.

The study population of 624 individuals had a mean age of 22.4 years, with the oldest individual being the battalion commander at age 39. Individuals ranked from lieutenant colonel to E-1 with 4.8 percent being officers, 95.2 percent being enlisted personnel.

Racial composition of this group included 74.1 percent white, 18.5 percent black and 7.4 percent other racial groups, including Spanish-American, Mexican-American, Indian and Oriental ancestries.

Approximately 19 percent gave a history of having been in Central America previously, and 7 percent gave a history of having been in the West Indies or South America previously. Three percent gave a history of being in Africa, the Middle East or the Indian subcontinent previously.

This interprets to mean that approximately 70 percent of the group that was being studied gave no history of prior exposure to an endemic area.

In January and February 1977, individuals presenting

with dermatologic problems following the exercise were evaluated. Cultures for leishmaniasis were obtained from all suspicious lesions and punch biopsies were, also, obtained from the more suspicious lesions.

A total of 30 cultures were performed, and 13 biopsies were done. Using positive cultures as a definitive test for the diagnosis of cutaneous leishmaniasis, it was possible to identify 10 individuals with this disease. This yielded a clinically apparent attack rate then of 16 per 1000 per two and one-half weeks of exposure.

Biopsies have failed to reveal characteristic organisms, and in general were usually non-specific but consistent with a diagnosis of cutaneous leishmaniasis.

The majority of these cases have begun treatment at the Walter Reed Army Medical Center with Pentostam, but it is too early to determine the real prognosis, since many have only recently been started on the treatment regimen.

Now, realizing that many of you may have specific questions regarding these 10 cases that were diagnosed, I have summarized their demographic and clinical information on the handout in front of you.

I would, also, now like to show you some pictures of at least five of these early cases to give you an idea what the early signs of this disease are like.

The first case is a 26-year-old E-5 who is study

number 075 on your handout.

The site of the lesions actually were the right hand and the left thumb, not the left hand or the right thumb as described on your sheet here.

May I have the next slide, please?

Here is a closer view of the same lesion.

Next slide, please?

The next case is a 30-year-old E-6 who developed a lesion on his right knee.

May I see the second slide, please?

This is number 293 on your sheet.

The third case is number 334 on your sheet.

Next slide, please?

Again, another lesion on the knee, a 22-year-old E-4.

Next slide, please?

The next case is number 340 on your sheet.

Next slide, please?

He developed lesions on the left lower leg, both across the skin and as well as on the calf.

Next slide, please?

This is the same individual. This is his calf.

The next case is number 383 on your sheet who developed lesions on his back which appeared to be more papular than anything else, no real ulcerations. He actually

had these lesions extending over his back and across his shoulders, a 40-year-old E-7.

Next slide, please?

This is a closer view of those same lesions. It should be reiterated that all of these cases were detected very early in the course of their illness, approximately one to two months following exposure in Panama.

Slides off, please?

The 10 cases that you have in front of you have had positive cultures obtained. In the last column of your handout, you can see it says pre- and post-deployment sera. These will be discussed by Colonel Diggs, and what we are referring to here is the indirect fluorescent antibody test.

What is especially interesting in the occurrence of disease is the occurrence by units. Five of the 10 cases belonged to B Company of the 1st of the 325th. Yet, if you look at the second table, you will see that the number of participants from B Company is not substantially greater from the other groups. Reasons for this high incidence in this one company are not clear at this time, since preliminary information that has been supplied to us from the Battalion Headquarters, as well as the Training Center suggests that there were no differences in the training. However, companies and platoons did tend to train together, and it is possible

that greater exposure did occur in this one group.

Please, also, note that the majority of the lesions seen were below the elbow and below the knee. One individual had positive lesions on his back.

As you review the photographs of the group in training, it will be obvious that individuals do remove their shirts and roll up their sleeves in tropical climates. Why we have not seen any lesions on the face or the neck is uncertain, but this may be due in part to the manner in which insect repellent and camouflage paint is being applied.

As a final note on the occurrence of disease in this group, let me just mention that orthopedic and dermatologic foot problems were the most common problems.

During the last four days of training, however, during the major battalion exercise, six individuals in this group developed fever of unknown etiology. Temperatures ranged from 102 degrees to 106 degrees with accompanying symptoms of malaise, myalgia and headache. Few respiratory complaints were noted.

One soldier was hospitalized at the local Cocosolo(?) Naval Hospital for several days. It was impossible, of course, to collect specimens from these ill individuals in the field. So, specimens were collected upon their return to Ft. Bragg. However, many of them were well into the convalescent phase by that time.

Specimens included stool, throat washings, urine and blood. No viruses were isolated, and paired sera were run for dengue, Venezuelan equine encephalitis, histoplasmosis and several of the sandfly fevers with no suggestion of an etiologic agent.

A malaria workup on the ill soldier that was hospitalized in Cocosolo was negative, and no relapses of illnesses have occurred in any of the six individuals.

In summary then over the last two years a total of 20 cases of cutaneous leishmaniasis have been diagnosed in Ft. Bragg personnel. Ten of these cases have occurred in the group under study, yielding a clinically apparent attack ratio of 16 per 1000 individuals deployed for two and one-half weeks of training.

Definitive diagnosis rested on positive cultures for organisms, since biopsies of lesions were often not helpful. It appears that the attack rate may be substantially higher for participants in the Brazilian jungle training course.

Specific locales of greatest exposure in Panama have not yet been identified, although there does appear to be a higher incidence of illness in this one company that I have mentioned, suggesting greater exposure in particular locales

Are there any questions?

DR. BENENSON: Dr. Farah?

DR. FARAH: Were direct smears done from these lesions?

MAJ. TAKAFUJI: Sir?

DR. FARAH: Direct smears?

MAJ. TAKAFUJI: From which?

DR. BENENSON: From the lesions.

MAJ. TAKAFUJI: Were direct smears done? No, they were not done.

DR. BENENSON: Dr. Gunning?

CAPT. GUNNING: You alluded earlier to a case that had been resistant to several courses of Pentostam and then resistant to Amphotericin. What was the total dosage of Amphotericin?

MAJ. TAKAFUJI: Major Hendricks, maybe you could comment on that. I think you know more about that case.

MAJ. HENDRICKS: He received 291 micrograms?

SPEAKER: 581 milligrams.

MAJ. HENDRICKS: Milligrams before his creatinine level reached a point where it was thought better to take him off it.

DR. MARSDEN: Marsden. So, it was chemically regarded then as an effective course?

MAJ. HENDRICKS: That is right.

DR. BENENSON: Dr. Nowosiewsky, you had a question?

COL. NOWOSIWSKY: Yes. Ernie, you mentioned that we saw those 10 cases that you presented as part of this study. Now, I know that you had two additional battalions participate in training, one that went in January and one that went in February. Would you indicate what was the incidence of cases in those units and also, how does your surveillance program differ from the unit that you are studying and to the one that you just provide with team surveillance?

MAJ. TAKAFUJI: There is no doubt that the group that we studied was under closer scrutiny than any other group that had been studied previously. The two groups that Colonel Nowosiwsky alluded to that have gone down to Panama since our group has gone down, we have not seen any cases of cutaneous leishmaniasis in those groups as of yet, but it should be remembered that the surveillance on these two groups is based on the individuals coming to their troop clinics with dermatologic problems.

The physicians and physician's assistants have been briefed on the problem,and they are referring questionable problems to the dermatology clinic, but it really rests on the individual coming in with a problem.

It is logistically impossible for us to do a thorough screening on all of these groups that have been down there.

DR. BENENSON: How many of these 10 men reported sick call under their own power?

MAJ. TAKAFUJI: None of them did.

DR. BENENSON: None of them. They were all picked up on your screening?

MAJ. TAKAFUJI: That is correct.

DR. BENENSON: Not even the one with the leg lesion?

MAJ. TAKAFUJI: That is right.

DR. BENENSON: Any other questions? Dr. Simpson?

DR. SIMPSON: Dr. Takafuji, what was the total follow-up period? I missed that in your discussion.

MAJ. TAKAFUJI: The group was deployed on the 19th of November. They returned on the 10th of December, and we have been following up this group ever since, and we plan to follow them up until this third bleed which will be in two weeks.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: The Group B as you commented is extremely interesting, with five members in the one group. I wondered whether these five people had stopped, you know, somewhere in the forest and had a smoke between the hours of 4 and 6 in the evening, you know. It is the obvious thing to go for, these microhabitats of the Phlebotomus. Were these five ever together between 4 and 6 and stopped in

the forest?

MAJ. TAKAFUJI: Let me just mention that three of these five belong to the same platoon. Two of them are black. One of them is a white individual. As far as we can determine, and I just got through talking, in fact, to these three individuals yesterday, as far as we can determine they train together. So, it is just impossible to determine where their exposure did occur.

DR. BENENSON: Dr. Walton?

DR. WALTON: In our 1965 study of a similar outbreak, if you will, in troops, we noted that the type of activity was very much associated with the incidence of infection and most of these cases came from what was then called the ATT and now ARTIP(?) the army training test for small units in which they were harassed constantly by a small aggressor force, and when we have almost a battalion size unit in the field 90 percent of the cases, if I remember correctly, but anyway the great majority of the cases came from the aggressor units which moved about during the whole exercise and probably never separated more than a few hundred yards from the main body of troops. So, actually the place of exposure was very little different. I believe that the key to the difference in type of exposure was that these men lay in concealment, in ambush. They lay down on the forest floor in a leaflet or tree buttresses without

moving during the period of time.

Dr. Marsden related to the period of activity, greatest activity of the sandflies. However, if you give them exceptional opportunity to bite, lie down in leaflet or in quiet during the day, they will take advantage of that opportunity. I think that this is the type of question you might very well ask in some of the other units. Was someone on aggressor duty? Were they on a night ambush, this sort of thing, I think is more pertinent than whether it is navigation or this sort of thing.

DR. TAKAFUJI: Nine of these 10 individuals did participate in this exercise that I described during the last week where the whole battalion went out, but they really did both things. In other words, they were both aggressors and defenders, you might say. The course has changed a little bit in their emphasis on certain areas and so forth.

What they do is they divide the group that comes into the school into approximately three to four groups, but they all cover the same areas, just at different times, but they all participate finally during the last week in this one battalion exercise.

We have thought about this, asking this type of question, but we haven't really found anything to indicate the ones that were more sedentary being the higher risk.

DR. BENENSON: Dr. Neva?

DR. NEVA: Neva, NIH. I wanted to just ask about your recovery of the organisms. Apparently then every one of the 10 patients who had clinical lesions you were able to recover an organism?

MAJ. TAKAFUJI: Yes, sir.

DR. NEVA: Then, I wanted to ask was that done at Ft. Bragg or were they all sent to Walter Reed? Was it the same group doing the culturing all the time and were they all from biopsies? Were the cultures from biopsies?

MAJ. TAKAFUJI: I mentioned the questionnaire that was distributed. A questionnaire was distributed at the time of the second bleed, and one of the questions that was asked is do you have any sores at this time or have you had any sores since returning from Panama that was consistent with the disease leishmaniasis, and what we did was we screened these positive answers, had them all come in and had them evaluated again a second time, and the more suspicious lesions were cultured.

Those are the 30 cultures that I alluded to earlier.

DR. NEVA: Then are there some lesions that haven't been biopsied?

MAJ. TAKAFUJI: Yes, there are.

DR. NEVA: There are. Okay. In other words, there may be some additional cases.

MAJ. TAKAFUJI: There may be some additional cases .

That is correct.

DR. NEVA: But the ones that were biopsied were all, you got positive cultures from all of them?

MAJ. TAKAFUJI: The ones that were biopsied revealed non-specific changes, in other words, inflammatory responses and so forth.

DR. NEVA: You are misinterpreting biopsy from -- I don't mean histologically, I mean by culture. Where do you get the material to put into the culture, from a biopsy or from a scraping or what?

MAJ. TAKAFUJI: No, these were done by aspirant which Major Hendricks will go into in more detail later on this morning. I purposely did not get into this because he is going to be discussing this.

DR. BENENSON: Okay, we will defer that question then since it is going to come up.

Dr. Burke?

COL. BURKE: Burke, from Walter Reed. I would like to refer you to what Dr. Walton and Dr. Marsden have spoken to on the occupation of an individual. In my experience in Panama I had one especially that was pertinent. He ran a switchboard. He moved from a road less than 50 yards away, set up his switchboard, stayed there for two days. So, we knew exactly, and he had never moved away from that area except from the road to the switchboard, and so it is

very micro oriented to where the individuals are and this was on the Pacific side of the Canal Zone.

Two other cases were two MP's out for a walk, and they did not want to leave because of falling off of a cliff after it got dark, and they camped in one specific area or slept on the ground was all they did, and again they came down with multiple lesions, all isolated and all this.

DR. BENENSON: Any other comments on Dr. Takafuji's presentation?

Dr. Marsden?

DR. MARSDEN: I just wanted to ask how many of these five people were smokers?

MAJ. TAKAFUJI: I don't know. We did not ask that question.

DR. BENENSON: Dr. Simpson has a couple of slides. Any other point of discussion while Ernie is up there?

Okay, thank you very much.

Maybe I do have another question. I am still not clear. You did not physically examine all 600 men. It was purely those who said that they had some sort of a sore on their body.

MAJ. TAKAFUJI: We examined all the individuals that we bled. Six hundred and twelve of the 624 individuals were examined and also bled.

DR.BENENSON: But then you confused me by saying

that those who were bled were asked if they had sores, and they were called back.

MAJ. TAKAFUJI: At the time of the bleeding a questionnaire was distributed, and it asked the question whether they had sores, and the ones who answered yes were then screened again, were pulled back in --

DR. BENENSON: For a second physical screening?

MAJ. TAKAFUJI: -- for a second dermatologic evaluation. That is correct. At that time cultures were done on the more suspicious lesions.

DR. BENENSON: Does that mean the first screening had missed the lesion?

MAJ. TAKAFUJI: The first screening only detected the presence of a lesion. In other words, the questionnaire asked whether there was a sore. A physician did verify that there was a sore, and if there was a sore then he was called back. He was put on a list, and this list included 30 individuals at the end, and these 30 individuals were then called back in and actually examined in considerable detail.

DR. BENENSON: Thank you.

MAJ. HENDRICKS: There is still some controversy over this. They were completely examined at the time they were bled. Anyone with a suspicious lesion or in most cases they claimed they had a lesion or whatever, were then rounded

up about a week later and cultured, but there were a couple of cases where the individuals did not know or did not claim that they had a lesion or a papilloma. It was found by the examination.

DR. BENENSON: All right. So, in other words, trying to get this straight, they came in for their bleeding, and they had a total body physical examination. If they had a lesion they were asked to come back a week later. If the lesion was not manifest and in their questionnaire they said that they had a lesion they were brought back. These were 30 people, 30 men who then were cultured, and of those 30, 10 were positive. That is the picture?

MAJ. TAKAFUJI: Yes, sir.

DR. BENENSON: Okay, thank you.

MAJ. TAKAFUJI: Oh, I should, also, mention that these individuals were asked about the presence of dermatologic problems before they went to Panama, and this was documented also, so that we wouldn't have any confusion in terms of source.

DR. BENENSON: Frank, are you clear?

DR. NEVA: No, I am not. In other words, then, 10 of the 30 that were cultured were positive?

MAJ. TAKAFUJI: That is correct.

DR. NEVA: There were 20 that were cultured that were negative.

DR. TAKAFUJI: That is correct.

DR. BENENSON: Tom? Dr. Simpson?

DR. SIMPSON: While we are loading these slides, or are they loaded? I would like to reinforce the point that Dr. Marsden made, that was reinforced earlier by Colonel Walton that probably lying doggo in the microhabitat of the Phlebotomus may be the responsible key to this.

We had a patient who was seen in 1968, I believe, in the Army Medical Center in Okinawa. This man had had training in the Sherman Rain Forest and had gone to Vietnam and had been picked up with lesions two and one-half months after the training period. At that time they were resolving to a large degree, but in the laboratory at Okinawa we were able to identify the organisms from the margin of lesions. So they were confirmed lesions, and I thought it would be of interest to show you this very quickly, the photographs of this man's lesions.

Could we get the lights off?

These are like lesions that Dr. Takafuji just showed, and we can go quickly through to the end one. Hear is one on his ear, but if you will hold that for a moment, we put this man in his customary sleeping position. He said that he always slept like this, lying always on this side, and if you notice, every one of the lesions are exposed. So, it may be that lying with one part of the body exposed

is the key to it, and it brings to mind the one study that I remember that came out of the Sherman Rain Forest from the military there a number of years ago where there was an anecdotal account of a soldier who was issued antimalarial including gloves, and this particular person was issued two left gloves, and the area on the wrist of the hand that had the malfitting glove was the one where the single lesion occurred.

DR. BENENSON: Thank you very much for the diversion, Tom.

Colonel Diggs is next on the serological studies on these cases.

COL. DIGGS: What I thought I would do is describe to you briefly how we went about setting up the test and then again briefly describe the data that we obtained on the first and second bleedings of the men who were exposed in the Canal Zone.

The sera were studied using a fluorescent antibody procedure based on that described by Walton, and the first slide will just give you an overview of the procedure as we did it.

The antigen was Leishman braziliensis, a Panama strain amastigotes from axenic culture, stored in 20 percent glycerol at minus 70, and the slides prepared and the organisms formalin fixed on the day of the test. The unknown

serum was diluted 1:16, applied to an antigen slide for 20 minutes, then washed six times followed by anti-immunoglobulin which was goat antihuman mixed globulins 1:150, with .1 percent Evans blue, 15 minutes and then washed six times again.

The sera were set up for the test by an individual who then divorced himself from the procedure and the readings, and then the slides themselves were examined for fluorescence by two observers who compared notes only after they had read.

Next slide?

To tell you how these were graded if the pattern showed that all organisms fluoresced by both observers they were graded then as 2 to 4+ depending on the intensity of fluorescence, and the interpretation was clearly reactive.

If 50 percent or fewer organisms fluoresced they were considered non-reactive. There were several grades here, negative, with no visible fluorescence, 1+ which was roughly 50 percent of the organisms fluorescing and then plus or minus in between.

If two observers disagreed, then we recorded this as weakly reactive and in the data that I will discuss I have lumped reactive and weakly reactive together.

As you will see, this results in that it turns out it essentially doubles the rate of non-specific reactors but it does, we felt, ensure that we would be looking at maximum

sensitivity.

Next slide?

This is simply the antiglobulin titration to show you what we encountered with one normal and one positive control serum. There was a little bit of non-specific reactivity at 1:4 of antiglobulin and 1:8 of the normal serum, and this disappeared in this particular serum at 1:16 of the serum, and you can see that reactivity in this particular positive control serum persisted with high dilution of antiglobulin.

Next slide?

This is simply a serum titration showing that even with this particular positive control reactivity fell off at 1:64.

Next slide?

Now, the variables that we studied in a very, I might say, rapid fashion, included these. We looked at fresh versus frozen slides versus bulk frozen organisms which were then used to prepare slides.

The only differences encountered here were a tendency for the more even distribution of organisms with the bulk frozen material, and this is why it was chosen. Otherwise we encountered areas of the slide that were highly fluorescent; other areas that were negative.

The counterstain,we obviously were able to visualize

increase in red counterstain as we increased Evans blue, and we chose .1 percent, which I believe was also used by Walton.

Fixation. We compared unfixed versus 2 percent formalin and saw no difference. We stayed with the fixed material.

Buffers. We compared barbiturate and phosphate and saw no difference, and we chose phosphate.

Protein additive. We looked with and without bovine serum albumin and we saw no difference. We chose to perform the test without BSA.

Next slide?

Now, this is one experiment of two or three that were performed prior to initiating the study to look at specificity, and these were sera just pulled from the freezer and we encountered -- I won't go through these, but we encountered a high rate of false positives among sera from various protozoan and helminthic diseases and in this particular case only two of three of culture proven cutaneous leishmaniasis were positive.

At this juncture although we had some reservations about proceeding, with the press of time we felt that we should proceed, and we did go on with the testing of the study sera.

Next slide?

Now, these are controls that were actually run

concurrently with the Ft. Bragg sera. There were 14 assays in all, and these are pooled, and as you can see we encountered about the same false positive rate but the difference here being that many of our putatively normal sera showed reactivity as well with these conditions, and most of the culture applies to proven cutaneous leishmaniasis were in fact positive.

Next slide?

This summarizes the actual data on the sera from the study. This can be looked at in a number of ways. I have chosen to do it first by the total number of reactive sera. As you can see, pre-exposure there were 21 percent positive. Post-exposure there were 22 percent positive. If one then looks at conversion rates non-reactive to reactive, this was 12 percent.

As a control the reciprocal, that is reactive to non-reactive is listed, and it was identical.

Sixty-seven percent were non-reactive to begin with and remained that way. Ten percent were reactive on both pre- and post-exposure.

Now, if one takes conversion rate and as the most specific, if you will statistic to look at, in the next slide in looking at the culture data which you have, actually you have before you, only one of the culture positive individuals were converted serologically. This is 10 percent.

This compares with 12 percent of the whole group or 13 percent of those which were either culture negative or which were not cultured because there was no indication for it.

Based on these findings, next slide, we must conclude that under the conditions that we tested these sera it is not useful for screening of individuals exposed in this particular time frame.

Any questions?

DR. BENENSON: I have a lot of questions. Who wants questions and then discussion? Any questions? Brice?

DR. WALTON: I am almost going to ask for equal time.

DR. BENENSON: Are you defending the Walton test?

DR. WALTON: No, actually you referred to it as our procedure. I don't even recognize it. There are certain basic differences. I am not being facetious, but I think there is something very basic here and that is you remember many people tried serologic tests for cutaneous leishmaniasis before with very little success.

The test that we have had very good luck with is based on a cell wall reaction; that is the key to the whole thing, and I got the first clue when you talked about weakly positive. We don't have such a thing the way we read the test. We do not use 1, 2, 3, plus fluorescence which gives you usually looking at cytoplasmic fluorescence, if you look

at cytoplasmic fluorescence you are going to have a lot of false positives and cross reactivity. If you look only at peripheral staining, that is cell wall reaction and you make your cutoff point if 50 percent or more of organisms are stained, then your reproducibility of a given serum is increased tremendously, and you get rid of about 90 percent of the grass(?) I think that is very basic.

Then you mentioned the organisms were obtained from axenic cultures. I assume you are talking about promastigotes which were grown and then converted into ground form in insect culture media with fetal calf serum? That is right? Okay.

We took a look at this before. Some lots of this type antigen were very usable. However, I have not been completely convinced that these are physiologically amastigotes equivalent of intracellular form. They have a lot of resemblances and in some cases antigenic similarity, but I was never convinced that we could control this to where we could call these amastigotes for this purpose.

So, if you do not use intracellular amastigotes for antigen, you do not look at the cell wall fluorescence, it is a different test.

Then I, also, noticed that you use a 1:16 cutoff. Now, the cell wall fluorescence test is not very sensitive. It is not the best serologic test, and that is for sure, and

one of the shortcomings is that it is not sensitive. So, you will lose quite a few positives if you don't use 1:8. We felt we got the bimodal curve and we can separate fairly well at 1:8. So, I think that would increase your conversion rate tremendously if you could go to that.

I, also, notice you use 2 percent formalin fixation. We use 1 percent. To what extent this denatures the cell wall, changes the reaction, I don't know, but this is another divergence from the original procedure. Also, the storage in glycerin I question. I think that you might very well block some reaction sites with that.

Okay, I have had my equal time, but I think that that in my mind is really the question. Are you looking at cell wall reaction? Because I think that this is the one thing right now in our very primitive state of the art that we can separate from this big mozaic of antigens. We have got something that is of diagnostic value.

DR. BENENSON: Carter?

COL. DIGGS: I think there were five comments and questions all jotted down here. As far as the cell wall fluorescence is concerned, I think that is what we are looking at. I mean it is a very, I think, difficult thing for you to document on paper what you are looking at for us to interpret, but to the best of my knowledge, and I think, Larry, you have seen both laboratories. You have seen the

preparations in both laboratories, that is what we are looking at. You know, we are looking at Evans blue counter-stain in the center of the cell with intense screen fluorescence and a good positive on the outside. I think that is something that we will have to talk about in more detail and compare notes about that. It is a very subjective thing.

As far as the amastigote or pseudo-amastigote situation, we elected to go this route because of the data which is available indicating that they are comparable and Larry, do you have your data that we could refer to? Well, perhaps it is not important to do that now, but this was on this basis that we went that way. Whether or not there is a batch dependency or not is something that obviously we could not control for.

We simply did not have time to go the virile route if the truth be known, but even if we had had the option we possibly would have chosen to go this way. The 1:16 cutoff versus the 1:8 cutoff, this is a very difficult thing to decide on when you are setting up a test in a different laboratory. You may be entirely right that 1:8 is better than 1:16.

The 2 percent formalin was an error on our part. I apologize. The storage in glycerin, as I said, was done after some frustration with dealing with slides that were

very non-homogenous and really we found it difficult, impossible, really, to grade a slide because it might be 3+ in one place and plus or minus in another, and finally you may be entirely right that all of these things may be crucial. I think we should compare notes in great detail.

DR. BENENSON: Dr. Farah?

DR. FARAH: We have had some experience with the immunofluorescent test for Leishmania. We agree that we are certainly looking at a cell membrane fluorescence. We have tried to look at the antibodies that are responsible for this reaction, and in a variety of ways that we have tried to look at this we were able actually to demonstrate that there are, at the most, three antigen antibody systems and the three antigen antibody reactions seem to be similar, whether we use, let us say, antibodies produced by immunization with whole parasite, promastigotes or whether the antibody comes from individuals who have had an actual infection, let us say the mouse for instance or the human, and the antigen antibody systems seem to be the same regardless of whether you are using as your immunogen an amastigote or a promastigote.

At least with the system that we have used we have been able to show these three antibodies, and so the question is whether it is not possible for one to use immunofluroescence using promastigotes which may be easier to obtain and which

could very easily be stored in a frozen fashion and on slides already prepared, fixed with alcohol and used for that purpose.

We have not, as I said, been able to demonstrate much difference between using promastigotes and amastigotes.

The second thing is that at least in our experience, and now, I come from an endemic area with leishmaniasis, and therefore our cutoff point in normal sera was 1 in 40, anything above 1 in 40 and now whether we used rabbit sera as normal rabbit sera as control or human sera as control, we were unable to differentiate the positives below 1 in 40, and our cutoff point, therefore was at 1 in 40.

I wonder if you compared promastigotes with amastigotes in this test, and we find actually a good correlation between positive cultures or smears or positive histology and the immunofluorescent test done this way.

DR. BENENSON: Dr. Walton?

DR. WALTON: Just to clarify one thing, are you talking about human sera, diagnostic sera or are you talking about experimentally induced antigen by injection?

DR. FARAH: Both actually.

DR. BENENSON: Dr. Neva?

DR. NEVA: There was one feature of Colonel Diggs otherwise pessimistic report that I found very refreshing, and that was the person reading the slides did not know what

they were. I think this is absolutely essential if you are going to have any kind of objectivity to a serologic test, and I think you just have to call them as they are, and this is what you get with this kind of a system. I don't think this is always adhered to, and it is so easy to be subjective when you have a patient, and you know you have got a lesion or you have got a positive culture, and you take his serum and you do an IFA test; you see different things in that than when it comes from a tube with a label on it or a number, and you don't have any idea what it is and when you test a lot of other sera, too, from patients with other kinds of diseases.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: Professor Couver who does a fluorescent antibody test for us in Brazilia has recently in the last six months switched over to the amastigotes. We cannot grow amastigotes. We don't have a tissue culture system. We get them out of a hamster node, but they make quite good antigen and clean them up, and all our titers have gone up one tube, you know. It has improved our fluorescent antibody test, undoubtedly using the amastigotes.

DR. BENENSON: Carter, Dr. Farah asked whether you had compared the promastigote and the amastigote, and I did not give you a chance to answer his question.

COL. DIGGS: No, we have not. I can relate very

briefly the little that I know about this. Ducksberry and Sodoon tried promastigotes some years ago and concluded that although there was some reactivity that they did not provide a suitable antigen for protein screening or clinical use.

I think I am correct in saying that there has been experience of Dr. Walton that promastigotes are not suitable. That is about as far as I can go at the moment. These people are better qualified than I am.

DR. BENENSON: Dr. Walton?

DR. WALTON: You do see reaction using promastigote antigen. There is no doubt about that. In certain patients you get quite good reactions, but the problem is we could not repeat our titers on the same serum in the same patient with the degree of reliability that we get with amastigotes. For us that was the key, and we, also, had much more trouble with false positives with promastigotes.

COL. DIGGS: I would be curious, Dr. Farah, your patients are what type of clinical manifestations?

DR. FARAH: Cutaneous leishmaniasis.

COL. DIGGS: Cutaneous, oriental and tropical, and you find that you can use promastigotes as the antigen in immunofluorescence?

DR. FARAH: Yes.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: One of the points that Carter

pointed out at the end in the final concluding statement was it does not account for false positives, but these patients were four to six weeks post return from the infected area with only two and one-half week exposure. So, it was very early infection when the sera was drawn. That may account for very few conversions.

DR. BENENSON: One out of five were positive before they ever left. That is what the problem would seem to be. I mean there was not much room for conversion.

MAJ. HENDRICKS: I tried to qualify that by saying that that did not account for the false positives or whatever, but the conversions that may account for the fact that very few had conversions.

DR. BENENSON: Interestingly in the 10 sera, three of them were read as doubtful, I guess. They are a plus in parentheses in the presera, and they cleared up by going down there and developing an infection. So, there is a problem there.

Any other discussion on the serology?

COL. DIGGS: I would like to ask one more question. Dr. Marsden, you switched from what to what?

DR. MARSDEN: We switched from promastigote to amastigote, but the amastigote did not come out of culture. It came out of the hamster node.

COL. DIGGS: Oh, I see.

DR. BENENSON: Any other questions or discussion? Thank you very much, Carter.

Major Hendricks, you are listed for 20 minutes. We have been riding a little bit late somewhat deliberately on my part because I want to bring these things out as we go. Duane, you list our coffee break to begin in 10 minutes. Is there anything critical on that?

LTC. ERICKSON: No, there isn't. I have a discussion period there which --

DR. BENENSON: No, we have been using the discussion period deliberately.

LTC. ERICKSON: So, we may come out with 15 minutes or something.

DR. BENENSON: All right. So, we go ahead with the next presentation. All right. Please, Major Hendricks on the culture techniques?

MAJ. HENDRICKS: To begin this presentation I would like to start off with the most basic question, and that is why do we use culture techniques as a means of diagnosis with cutaneous leishmaniasis. The reason actually is fairly simple and results from a process of elimination of commonly used diagnostic techniques for other diseases.

The techniques used with many diseases are just not as obvious with leishmaniasis or the state of the art just has not yet reached that point of development where they

can sufficiently be relied upon with cutaneous leishmaniasis.

Clinically this infection must be distinguished from tropical ulcer, eczema, impetigo, sporotrichosis, South American blastomycosis, syphilis, leprosy and tuberculosis of the skin to name but a few.

Histological examination of biopsied specimens of cutaneous lesions from many parts of the New World have a low diagnostic sensitivity as few parasites are present. This point was previously mentioned by Major Takafuji in his discussion of the Ft. Bragg investigation in which none of the patients shown to be culture positive have had Leishmania organisms demonstrated on biopsy examination, histological examination of biopsied material. Very few laboratories, civilian or military offer serological test for the detection of this disease. The data presented by Colonel Diggs seemed to indicate that the techniques used in this particular study are not sensitive enough to detect infection as early as two months post-exposure.

Skin test antigen used in many parts of the world to assist in a diagnosis of this disease is not available in the United States. Evidence from the literature and from personal experiences indicates that neither serological testing nor skin testing is of little or any use to determine if a patient has successfully been treated or as a diagnostic tool if future suspect lesions occur, as both remain positive

for years in the majority of cases.

Because of the limitations of the successful diagnostic techniques offered for this disease, many of the therapeutic treatment studies of the past and many clinics today still rely on visual recognition of re-epithelialization of the lesion site as evidence of a successful treatment of cutaneous leishmaniasis.

This latter point is demonstrated in the next series of slides. I want to demonstrate or to show you that a patient in which it was based on appearance of his lesion post one treatment with Pentostam this is the way the patient appeared at the clinic. Positive culture in Schneider's insect media confirmed the diagnosis of cutaneous leishmaniasis.

Next slide, please?

This was the lesion site one week after receiving a 10-day therapeutic course of Pentostam. Based purely on re-epithelialization of the site the patient was discharged from the hospital, sent back to duty. Seven days later the culture became positive, and the patient was brought back, rehospitalized and another course of Pentostam administered, 10 days again.

Next slide, please?

This was taken three months after the last course of Pentostam, and repeated negative cultures, the point being

that just on the basis of the fact that re-epithelialization of the original lesion site has occurred is not a sufficient diagnostic tool to say that you have cured the patient.

Slide off, please?

Faced with these circumstances and data, we feel that when the disease is suspected, cutaneous leishmaniasis the most reliable preferred method of diagnosis is the direct culture of the organisms.

In the drug treatment protocol currently under evaluation at WRAIR and RAMSEY that will be explained in more detail by Colonel Canfield, no patient is judged a successful treatment until at least two successive negative cultures have been demonstrated.

The life cycle of all known species of the Leishmania includes two morphological forms of the parasite.

Slide, please?

The flagellated form found in the sandfly vector, called the promastigote and the ovoid or round form found intracellular in the vertebrate host, amastigote.

SPEAKER: We need a professional projectionist, obviously.

MAJ. HENDRICKS: Somewhere we went by a promastigote and an amastigote there.

All culture techniques used to detect the parasite involve removal of tissue from the vertebrate host, placing it

in a media in which parasites if present will convert to the promastigote form and hopefully multiply at ambient temperatures, 24 to 26 degrees centigrade.

Slide off.

Thus the usefulness of most media is related to its ability to induce the transformation of the parasite from the tissue inhabiting amastigote form to the flagellated modal promastigote and the subsequent multiplication of the parasite to sufficient numbers to be detected.

The diagnostic media described today use basic blood agar media and varying amounts of blood from a variety of sources to provide a media originally described as triple N media.

Other diagnostic media described include Manseur's and Tanaby's, both consisting of blood hemolysates and saline combinations and are used primarily for detection or work with visceral leishmaniasis.

Most of the military laboratories have such a low demand for leishmaniasis diagnostic media that few, if any keep it in stock as it soon is outdated or no longer of any value and must be discarded.

We have recently found that Schneider's insect media plus 30 percent fetal calf sera can be successfully kept at least two years in the lyophilized form at ambient temperatures, and as will be shown later this media is quite

useful as a diagnostic media.

The method of acquiring a specimen to be cultured can be quite important. If proper care is not taken, many cultures will become contaminated with either bacteria or fungi or often both from the secondary infections found at the site of the lesion and usually will rapidly multiply in the culture media and completely overgrow to the point that the culture system itself is of no diagnostic value.

A technique that Dr. Walton used successfully in Panama and which we use to our satisfaction is that of needle aspirations of the lesion.

After the lesion site has been thoroughly scrubbed with soap and water and then followed with an alcohol wash and allowed to air dry, slide, please, a small amount of sterile saline is injected into the raised edge.

Let us go on a couple more, Duane, I think there is a picture of a lesion and a needle aspiration technique.

This is a line drawing of the procedure, but the idea is to inject a small amount of saline with a small syringe, usually a disposable tuberculin syringe on the outer margin or intact margin of the skin, inject a small amount of sterile injectable saline in the site.

Next slide?

This is actually using it on the lesion, rotating, injecting a small amount of saline, rotating the needle

around several complete turns, letting it act as a biopsy punch, if you will, putting back pressure on the plunger and then slowly removing both the fluid, the saline that you have injected and any other tissue or patient's sera and blood that you can pull back into the plunger.

Next slide, please?

This is the Schneider's insect media with 30 percent fetal calf sera. It is quite clear, as you can see. So, it makes it easy to observe any movement in it if you use a inverted phase microscope.

Once you have removed the droplets of fluid from the vision site, then place them directly into the culture material and we usually use or in most cases we use a small amount of penicillin and streptomycin as a backup just in case some contamination or some bacteria did manage to make it into the culture process.

Slide off.

In 1973, while the Army still had a research unit in the Canal Zone it was found that several commercially available media used for the cultivation of insect cells in vitro were also very effective media for the cultivation of a variety of species in geographic isolates of Leishmania.

Subsequent investigations using these media demonstrated that they not only were effective for the growth

of large numbers of organisms but that they were, also, quite sensitive for the cultivation of the parasite from suspect lesions.

Slide, please?

This slide represents data accumulated from some of the earlier cultivation work and is shown to demonstrate the growth characteristics of the particular medias that we are discussing.

The dotted line represents Grace's insect medium and 30 percent volume per volume of fetal calf sera. The solid line represents Schneider's Drosophila medium plus 30 percent fetal calf serum and the dashed line represents mammalian tissue culture media 199, plus 30 percent fetal calf sera.

Schneider's medium originally developed by Dr. Imogene Schneider of WRAIR was based on an analysis of insect hemolymph and was used by her to cultivate Drosophila, fruitfly cells in vitro, was selected as the medium of choice for the bulk of our work, as cultures remained viable for up to 30 days with this particular media.

Recently we have undertaken a comparative study to determine the sensitivity of various media in the detection of suspect cutaneous Leishmania patients.

Separate needle aspirates taken from suspect cutaneous lesions have been added to both triple N medium

made with 15 percent defibrinated rabbits' blood and blood agar base and Schneider's insect medium plus 30 percent fetal calf sera.

Today 99 such comparative cultures or lesions have been cultured with both media. These include suspect cases not previously seen, proven cases of cutaneous leishmaniasis and patients cultured after drug therapy.

To date 37 Schneider's culture media have become positive compared to seven triple N cultures. The shortest culture time for a positive culture in Schneider's media was 18 hours and this is all with human data patients; working with a laboratory infected animal we were able to get positive cultures in as short as six hours using the Schneider's insect media, actually had conversion from the amastigote to the flagellated promastigote form and could see the promastigotes thrashing around in the media.

The shortest time for a triple N culture with human patients to become positive was three days. The average time for Schneider's media to become positive of the 37 cultures that I mentioned was 7.1 days, while the average for the seven triple N cultures was 14.5 days.

We are continuing with this study. It was originally set up as a three-way comparison. We did Tanaby's media which has been described as being quite sensitive for the detection and growth of visceral leishmaniasis. As

I said, we have done 99 cultures of this three-way comparison to date. We have yet to get a positive culture in Tanaby's medium. We have used several recipes. We have asked Dr. Bill Hanson at the University of Georgia Vet School to send us some of his Tanaby's. He uses it as a detection method and growth for visceral leishmaniasis, Leishmania donovani Khartoum strain to be specific, but we have as of yet to acquire a positive culture using Tanaby's media on needle aspirate technique on patients from Panama and from Brazil.

Thank you.

DR. BENENSON: Thank you. Dr. Neva?

DR. NEVA: I would like to ask a question and then make a possible suggestion. In your comparative study did you start with the same inoculum? You referred to repeated needle aspirates in order to inoculate the different culture. One of the problems with this type of a study is you should start with the same inoculum. That is why if you took a biopsy, ground it up or otherwise pooled your needle aspirates and then from the pooled material inoculated equal volumes to the different cultures, then I think you would have a better comparison.

I don't doubt that this medium probably is superior to others, but I think that is really the only way you can make a decent comparison.

MAJ. HENDRICKS: We had 30 patients, and we had the dermatology clinic for one afternoon. We did not pool them, but the person that was handing forward the culture flasks stood behind me as I made the aspirates, and I did not know what culture media he was handing to me. I just made three aspirates for each media around the suspect lesion. When he would hand me a media tube, I would inoculate the inoculum into it.

DR. NEVA: The trouble with the aspirate technique is you are getting such a tiny amount of material and that is why I, personally, would prefer using a punch biopsy and then --

MAJ. HENDRICKS: Interestingly enough, we punch biopsied 13 of these 30 patients, and we picked the ones that we were most suspicious of to punch biopsy. We took half of those punch biopsies and ground them and added them to media as well, and only three of them became positive.

DR. BENENSON: Dr. Walton?

DR. WALTON: I am pleased that you had the chance to go ahead and do some more work with this liquid media and that your findings that you can keep it on the shelf for a couple of years. To have something when you need it, I think is very important. I had one question first and then a comment, if I may.

In your comparative study I missed what temperature

was the most effective in incubation in a liquid medium. Did you say 33 or --

MAJ. HENDRICKS: What temperature did we keep the cultures at?

DR. WALTON: Yes.

MAJ. HENDRICKS: We kept them at 24.5, as close as we could get it with the incubators we had.

DR. WALTON: The other comment I would like to make is very basic, and it has to do with a subject we have alluded to before, and that is the differences in Leishmania. The taxonomy, the classification of leishmaniasis is in a very pronounced state of flux right now, but one of the widely accepted differences in leishmanial strains is based on biological characteristics. One of these characteristics is how well it grows in triple N type media, and of the braziliensis group generally these do not culture very well. L. braziliensis pansmensis is one of the exceptions. It is the only one in, well, one of about two in the braziliensis group that can be successfully cultured. Braziliensis braziliensis the form that is the most hazardous from the standpoint of later development of mucocutaneous disease is very difficult to culture and we noticed this very early on in our work in Panama, as a matter of fact, and we alluded to it in the Camolar study that we were unsuccessful in trying to monitor the success of treatment because we

couldn't culture the organism in these patients at all. We found them in biopsies and smears, and the interesting thing in Panama and the Canal Zone is that of the two hundred and some patients, military patients that we looked at by culture and all these techniques, the last time I looked at the data, the great majority came from the Pacific side of the Isthmus, the empire range. In this group we had 100 percent success in getting blood agar cultures. In a much smaller number of patients from the Atlantic side in the Ft. Sherman area we had repeated cases of cutaneous lesions where we could demonstrate the lesions in which we could not culture. So, culture is not the definitive answer in making the diagnosis when you are dealing with braziliensis braziliensis, and this is something that you should always be aware of.

DR. BENENSON: Dr.Marsden?

DR. MARSDEN: Yes, I would just like to add to that, you know, that in our area of the Central Plateau of Brazil where there is probably the worst leishmaniasis in the world mucocutaneous leishmaniasis, we are not using your medium which I am sure would be a great help to us, but we are just using the routine NNN, but in fact in our work in the area we have dropped using culture in the sense that we have had so much contamination and such poor results. We are now doing smears from multiple punch biopsies and inoculation of the hamster nodes is our routine. We seem to

have more success with the hamster nodes. And of course, even in the hamster nodes, our strains grow extremely poorly, and it is impractical because you have to give the hamster six months a year, you know, to get anything. It would be very interesting, actually, to compare the development that you are studying with the hamster and the success rate in the hamster because many field workers are really using the hamster because hamster cleans things up so nicely in the nodes and the cultures in primitive field conditions that are constantly unsatisfactory.

MAJ. HENDRICKS: We feel that the lyophilized form of this may be very useful for field work because you don't need to reconstitute until you actually find someone you think that has a lesion and you can carry it around at ambient temperature, in a clothing pocket or something.

Concerning something that Dr. Walton mentioned, we have cultured positive -- I would not attempt to put a taxonomic name on it, but these were patients from Manaus, Brazil at the Brazilian General Warfare School. We have cultured four out of the four that have attended the school that we have been able to see had lesions, and we have cultured all four of them positive with this insect media.

There have been three or four cases out of this last group of 20 that Maj. Takafuji mentioned as coming from Ft. Bragg within the last two years in which we found a

considerable number of amastigotes at the site of the lesion and had tremendous difficulty. In fact, we have not yet successfully continued to cultivate culture of any of these particular strains.

You can see occasional promastigotes. In other words, the amastigote is converting to promastigote, and you do see movement in the culture media when you looked at it with phase microscopy, but we have not yet been able to get them to continue growth, and we have tried temperature variations. We have tried different concentrations of media. We have added whole blood. We have added human B type blood, and we have not yet had any success.

Now, there is something very similar to this. In fact, Dr. Peters in Liverpool who uses the DNA buoyancy isoenzyme test received some of this material that was described from very similar type cases in Northern Brazil, and Dr. Peters has called it Leishmania species. He has not seen fit to put a taxonomic name on it yet, either, and usually they are quite difficult to cure. One of the patients we will be talking about later that is a three time treatment failure with Pentostam and received over 500 micrograms of Amphotericin was one of these particular cases.

DR. MARSDEN: Where was he from, that patient?

MAJ. HENDRICKS: He was from Panama, sir.

DR. BENENSON: Dr. Neva?

DR. NEVA: Two more comments, one of them, it is apparent that you are aware of this when you use two different strengths of blood, but certainly it is known that if you use, say, 5 or 10 percent rabbit blood in the classical triple N medium you may not have as good success as if you used 20 or 30. We actually use 30 percent for our more enriched medium. Whether that would make any difference from your 15 percent --

MAJ. HENDRICKS: We have used 50 percent defibrinated rabbits' blood, and we have used 15 percent outdated human B blood.

DR. NEVA: The other comment I wish to make is something that I think Brice could comment on, and he certainly, I think, commented on this in the literature, and that is the persistence of organisms in a lesion that is otherwise healing may not necessarily by cause for concern that this is going to reactivate. We have noticed in several patients whom we have seen where you treat them, the lesion is epithelializing where you may or may not be able to feel little nodules within this area and on several times we have gotten positive cultures from these, and we have just watched the patient and not given any more courses of therapy and they have eventually apparently healed so that mere persistence of organisms does not necessarily mean that they are going to break down and give clinical activity.

MAJ. HENDRICKS: Along those same lines right now, sir, we have one that we have been watching for about 18 months. They have closed. They have reopened. They have closed. He has had three courses of Pentostam and one course of Amphotericin B and he still has open lesions that are culture positive.

DR. BENENSON: I think what you are saying, Frank, is there is a difference between Leishmania infection and leishmanial disease, and what we are trying to do ordinarily is cure the disease, hopefully, the infection, too, but we judge by the disease.

Any other questions? Dr. Marsden?

DR. MARSDEN: Such observations would always relate to the length of follow-up. For instance, we had a patient recently in Brazilia whose cutaneous lesions cured one and one-half months after therapy. Twenty months later he came back with the nodes full of granuloma. So, the duration of follow-up is very important. I think it unlikely that patient was reinfected.

DR. BENENSON: Dr. Walton?

DR. WALTON: Several years ago we had the opportunity to see some patients who were living in La Paz, Bolivia, at some 13,000 feet above sealevel where we were very sure there was no leishmanial transmission going on. There had never been any cases, and we encountered there four patients who had

previous exposure in the endemic areas in lower altitudes in Bolivia, and these patients, all four had espundia, that is mucocutaneous manifestations, and if I remember right one of them had 11 years, one 13 years, one 18 years, one 24 years with absolutely no manifestations, no indications that they were infected, and then evidently due to immunodepression from other causes one was pulmonary TB, one was senile debilitation, and one malnutrition they suddenly broke down into a fulminating espundia. So, quite possibly the military can take care of the case and leave the problem for the Veterans Administration some 18 to 20 years later.

DR. BENENSON: Maj. Hendricks?

MAJ. HENDRICKS: One other point, sir. This particular patient that I mentioned as a three-time treatment failure with Pentostam and also after a course of 500 milligrams of Amphotericin B, during his second course of Pentostam he was on 10-day convalescence back in North Carolina. This was during the wintertime. When he returned his original lesions were a particular case because he was a red herring for a long time. He had abraded himself on the right calf while on a night compass march under water. His leg was abraded under water by either coral or concrete or something. He was not sure which, and he had a small abrasion burn there from repelling on his right wrist from the helicopters, and since these were all traumatic injuries he was pushed

aside for a long time and was not seen.

After some six or eight months of lesions persisting he did make it to the dermatology clinic and eventually we did culture him, but between his second and third course of treatment he cut his left calf with a briar while he was on convalescent leave back in North Carolina, and when he returned the top of that abraded mark had enlarged considerably. We cultured it, and it was then positive, also for promastigotes, within a 10-day period of time.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: Yes, I just want to interpolate. I presume that we are going to discuss this unusual patient you keep throwing out, indeed, but I think we ought to refer to him as a patient who did not have a therapeutic course of Amphotericin B. I do not regard 500 milligrams as an adequate trial of Amphotericin B. So, I think Pentostam and Amphotericin B was tried.

MAJ. HENDRICKS: He did have what FDA allows us to give of Pentostam though, sir.

DR. BENENSON: I think we have gone far enough without coffee,and Duane would you rediscribe our itinerary?

Colonel Erickson raises a question of a physiological break for about five minutes and then let it ride that way instead of taking a coffee break. I would

prefer that unless someone wants to register objections. I think we have been dealing a little more deeply than the schedule called for.

Let us take a 5-minute break. Where are the physiological refreshment areas?

(Brief recess.)

DR. BENENSON: Let us go back to work. Next on the program is Colonel Canfield who will describe for us the therapeutic studies that I believe are currently underway.

Colonel Canfield?

COL. CANFIELD: Thank you.

First slide, please?

The first slide just orients us as we have already been hearing about this morning and shows the number of cases over the years. It goes back to 1953. Nearly all of these patients were seen and diagnosed in Panama at the Army Medical Research Unit there.

As you can see there was an increase in the early 1970's and a later increase more recently about which we have been hearing this morning.

In 1973, Dr. Walton summarized the results of treatment studies undertaken on many of these patients, and the results are shown on the next two slides.

Rather than reading this off, I will just pause here momentarily so you can study the slide. This morning

we are going to focus on --

DR. BENENSON: I think it would be better if you do read it because we cannot see it back here.

COL. CANFIELD: Let us move the table.

DR. BENENSON: Now, we can read.

COL. CANFIELD: I would like to concentrate on the two pentavalent antimonials, Glucantime which is a glumine(?) antimonate and Pentostam sodium stibogluconate, and will refer only to the 10-day course.

Initial treatment with a 20-day course of Glucantime gave merely an 80 percent cure rate and a 10-day course of Pentostam gave a 70 percent cure rate. These differences are not statistically different.

The next slide shows second and third course treatment. There is a small number of cases. The patients were not crossed over, and they were not done in a randomized style. This was a retrospective accumulation of data, and so we cannot draw any conclusions about the relative efficacy of drugs.

There appears to be a slight superiority of the Glucantime but we cannot draw any firm conclusions on this. I will compare the relative amounts of antimony in the two regimens in a minute.

As patients with leishmaniasis began to arrive CONUS during the last year following exposure at the jungle

warefare school, they were treated with Pentostam under the CDC IND. The results of these treatments are shown on the next slide.

Treatment failure was determined by culture. In three instances, although clinically improved, followup cultures are still being observed for possible growth and are designated on the slide by a question mark.

As you can see here, the cure rate by culture criteria was only 20 percent for the first course of treatment with Pentostam.

A second course was somewhat more successful, but there are two individuals who have not been cured with three courses, as well as Amphotericin B.

Two individuals were treated with Glucantime under special approval from the Surgeon General. However, we do not plan to do Pentostam, Glucantime comparative studies for two reasons. First, Glucantime is very difficult for us to obtain, and second, we have been unable to secure the preclinical and toxicological data necessary to support a clinical trial under FDA regulations.

The next slide shows the comparative quantities of antimony in the two treatment regimens. The manufacturer's recommendations are shown on the top of the slide.

As you can see, the dose of Glucantime is almost impractical, 20 cc's daily. Both Dr. Walton and we have used

only 5 cc's daily.

Despite this reduction in the daily dose, the quantity of pentavalent antimony still exceeds that recommended for a single course of Pentostam because of the longer duration of treatment.

You can take the slide off for a moment.

Let me recapitulate then our position of about six months ago. We anticipated one or two hundred cases of cutaneous leishmaniasis based on our preliminary guesstimates and although Glucantime seemed to be slightly superior, it was not proven to be superior. The drug was not available, and the slight superiority was thought at least to be simply a reflection of the dose of antimony. We, therefore, chose to concentrate on Pentostam.

Early results using the recommended treatment regimen though had been discouraging. We considered increasing the dose or duration of treatment,but could find no published data to support such an increase, and the preclinical data were of such quality and quantity as to make this an unsupportable course of action.

We did, however, have one additional piece of data that guided our actions.

In 1947, Otto had measured antimony concentrations after single IV doses 3 and 6 milligrams per kilogram of Astibanose(?) which is a diethyl aminoethyl salt of antimony

glyconate. The data were good enough for us to perform kinetic analysis using non-linear regression as shown on the next slide.

The data fit a two-compartment open model or a biexponential equation. The mean volume of distribution was about 13.9 liters. The distribution half time was 30 minutes, and the elimination half time was about 4 hours. As you can see, significant blood levels persist for relatively short portion of the 24 hours between doses and reach zero by the time the next dose is to be given.

Although therapeutic effect may be a function of peak drug level, it more likely is a function of the time/dose relationship. We, therefore, decided to modify the delivery of the drug so as to increase the time/dose relationship without increasing the total amount of drug delivered.

With the assistance of the CDC and Burroughs-Wellcome we prepared our own IND which was subsequently approved by the FDA. The clinical protocol was a cooperative venture between the Division of Medicinal Chemistry of WRAIR and the Infectious Disease Service at the hospital, at Walter Reed.

Arrangements were made for all Army personnel to be sent to Walter Reed for treatment.

The next slide shows the proposed treatment regimens for the clinical protocol. Patients are offered treatment according to the standard or manufacturer's

recommendation or allowed to volunteer for the investigational protocol. If they volunteer they are randomly assigned to Treatment A, B or C. A is again the standard regimen with drug being administered every 24 hours. B is the same total amount of drug delivered by constant infusion following a loading dose, and C is a compromise between the two with one-third of the dose being administered at 8-hour intervals following a loading dose.

We did not choose these as recommended or practical methods of drug administration. Rather they were chosen to test for the sustained levels, even if lower might be superior to intermittent levels. A revised formulation could be developed if sustained levels were found to be superior. It might be, for example, that the old preparation containing,the old oily preparation containing 54 milligrams per milliliter of antimony might be a better way to produce and continue effective blood levels.

The three regimens are illustrated on the next slide. This was drawn by hand and is slightly out of scale. The peak concentration with treatment A would be equivalent to about 43 micrograms per ml, and the sustained concentration with treatment B should be about 3.8 micrograms per ml. Again, though, the area under the three curves is equal because the total dose is the same.

During the proposed study blood is being drawn for

analysis of antimony concentrations in order to test the accuracy of this model.

The final slide shows the design of the study. As we previously mentioned, patients who volunteer for the investigational portion are randomly assigned to treatment A, B, or C. Failures from any of the three groups would be randomly assigned again to either of the alternative groups. Failure from the second course would be assigned to the remaining regimen.

This design ensures that the largest number of individuals will receive the best or most efficacious regimen. Thus far, eight patients have been treated under this protocol, and it is too soon to make any comments on the results. Cultures all thus far have been negative.

Slide off.

There is one final point on the protocol. Major Hendricks has adapted the culture system in order to quantify the response of individual patient's organisms to Pentostam. It will be, therefore, possible to estimate the relationship between blood levels achieved and minimum inhibitory concentrations in vitro.

For example, one study has been done that showed the minimum inhibitory concentration of one of the organisms to be about 2 micrograms per ml of Pentostam antimony, expressed as antimony.

It will also be possible to assess if treatment failure with a single course of therapy produces a variant organism with decreased susceptibility to Pentostam.

DR. BENENSON: Thank you. Questions? Dr. Schultz?

DR. SCHULTZ: How did you give the sustained levels of antimony in Protocol B?

COL. CANFIELD: Continuous intravenous infusion.

DR. SCHULTZ: For 10 days.

DR. BENENSON: How long each day each infusion?

COL. CANFIELD: All day.

DR. BENENSON: All day long.

COL. CANFIELD: All day and all night.

DR. BENENSON: How many veins?

COL. CANFIELD: It has not been a problem.

DR. BENENSON:You did not change the insert?

COL. CANFIELD: Every 48 hours.

DR. BENENSON: Okay, 48 hours at one vein site.

DR. MARSDEN: Do you do transaminases, urea before and ECG, do you follow these?

COL. CANFIELD: Yes, that is the usual laboratory tests. They have daily electrocardiograms, usual standard kind of checking for any investigational drug.

DR. BENENSON: Dr. Neva?

DR. NEVA: I would like to make a plea again, if you are going to be evaluating your results of these three

treatment schedules to pool your needle aspirates when you are culturing so that then,well, you are going to be culturing in only one kind of medium, I guess.

COL. CANFIELD: Yes, I think that that is a valid comment to test two different culture methods, but in terms of --

DR. NEVA: Okay.

DR. BENENSON: My question is what is failure? How do you define treatment failure?

COL. CANFIELD: We have arbitrarily chosen to define it as a culture positivity.

DR. BENENSON: Taken when?

COL. CANFIELD: Taken, there are either two or three cultures, two cultures after a course of treatment. The first one is about 48 hours and the second one is a week or two afterwards.

DR. BENENSON: And a failure is if you find organisms on the culture a week or two after termination of treatment?

COL. CANFIELD: Yes.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: I am sorry. I am still about fazed by it, but these treatment schedules, what is the interval between them? Supposing he fails on the first one, when will you elect to start the second?

COL. CANFIELD: With demonstration of culture positivity.

DR. MARSDEN: Which could be a variable time or is --

COL. CANFIELD: That is true. It could be.

DR. BENENSON: Major Hendricks, did you have a question?

MAJ. HENDRICKS: I just wanted to point out we are dealing three days after the last culture is made, the first culture made after the last treatment regimen and then again after a 10-day convalescent leave. So, it is three and 13 days when the cultures are made after the treatment.

DR. BENENSON: Dr. Schultz, what data do you people get on the Pentostam used around the country or is very little used?

DR. SCHULTZ: There is a fair amount that has been used. I just had my clerk analyze the data over the past 10 years, and there has been 138 doses of Pentostam dispensed during the past 10 years.

DR. BENENSON: Thank you, Colonel Canfield.

DR. SCHULTZ: One hundred and thirty-eight doses of Pentostam in 10 years' time. So, it is an average of 14 cases a year although this may not mean that there are 138 patients because some are repeat treatments. Of those 138, at least 45 were military cases. One of those was a

military dependent.

We don't know which service of the military contributed to those 45, but the proportion that is military of the total is increasing in recent years and all but one of the military came from Panama, jungle training.

DR. BENENSON: That is the total consumption. You have no data on the success of the therapy?

DR. SCHULTZ: No, unfortunately, I don't have any data on efficacy with me, but I would be happy to put it together and make it part of the proceedings of this meeting. I just have not had time in the past several weeks, but I do have some data on safety of Pentostam, part of which I just got over the phone a few moments ago, and we have analyzed 59 cases which received Pentostam, and I have a list of side effects here. I won't give all of them to you because they are numerous, but the most common side effect was pain at the injection site in nine patient, nine of 51 patients.

DR. BENENSON: These were all given intravenously?

DR. SCHULTZ: I cannot say for sure.

DR. BENENSON: You list, Colonel Canfield that standard therapy is intravenous.

COL. CANFIELD: Yes.

DR. SCHULTZ: In our protocol --

COL. CANFIELD: It can be either way.

DR. BENENSON: I see, you say either one. Okay.

DR. SCHULTZ: That was the most common side effect. The next most common was malaise and then the next most common occurred in several different signs and symptoms in three patients each, nausea in three, elevation of SGOT in three, vertigo in three. That is all.

In two patients each arthralgias, fever, headache, that is all, and in one patient each myalgia, abdominal cramps, epistaxis, weight loss, chest pain, anorexia, stomach ache, cough, weakness, tachycardia, elevated blood pressure, dyspnea, diaphoresis. Now, of course, these are a wide variety of constellation of signs and symptoms. I cannot vouch that they are all due to antimony.

DR. BENENSON: Colonel Canfield you have the degradation curve of the drug intravenously. Do you have any data on the intramuscular?

COL. CANFIELD: No. That is old data. It is 1947, and we will get our own intravenous data. I am trying to think if that same drug was not given intramuscularly in that study.

DR. BENENSON: Dr. Neva?

DR. NEVA: Let me ask kind of a naive question? Can you account for the pentavalent antimony all in the blood? Is it possible that it may be found in macrophage cells, stored in other tissue compartments?

COL. CANFIELD: Probably not. Certainly in animal

studies that they have done in which they have looked at recovery, it is nearly all excreted in urine within the first 24 hours.

DR. BENENSON: The thing I am wondering is if you put it intravenously, it is going right through the kidney. It is going to be excreted much more rapidly. If you add the complication of having to be picked up from a tissue deposition site that would give you a much, I would expect, more prolonged blood level, wouldn't it?

COL. CANFIELD: It would affect the absorption phase from the site, yes, and depending upon where it was given and the vascularity of the muscle, but a drug such as Pentostam which is so soluble in water, it would be picked up very rapidly. Yes, it would delay it and spread it out a little bit.

DR. BENENSON: But maybe only 30 minutes, is that what you are saying?

COL. CANFIELD: Yes, I would think no more than that. I am just guessing. I have not seen the --

DR. BENENSON: Dr. Walton?

DR. WALTON: I had one point and one plea since I have a precedent for making pleas in a study. The point is that there is quite a bit of evidence that there is distinct difference in susceptibility of different leishmanias to antimonial therapy, well, other drugs, too. The mexicana

type from Mexico and Central America is much easier to treat in general than particularly braziliensis from certain areas, and this is not well defined, but I think it is real, and I mentioned before we have some evidence that we have more than one thing in Panama. So, therefore, in your series maybe you are treating different bugs. So, if you, the plea is, put some of these isolates down and get the cryopreserved, get them in places like Liverpool or London, the only place they are doing biochemical taxonomy now to see if there is any difference, this might explain differences in case you have discrepancies.

One other point, I don't think Carter Diggs was very enthusiastic about serologic monitoring effects of treatment right now, but if you put some serum down in a freezer, I would like the chance to run some blind before and after.

COL. CANFIELD: Particularly the first point we would agree with, and I have a question. Has anyone ever looked at an in vitro dose response curve of drugs to determine the susceptibility in vitro?

DR. WALTON: The answer is there are probably a dozen or so papers published on this, and they have all been done with promastigote forms which is irrelevant.

The group in Liverpool started doing some of these things in their tissue culture system, but they ran into

problems. They had that dark sarcoma line that they are having problems with. So, I don't think anyone has done it.

DR. BENENSON: Dr. Simpson?

DR. SIMPSON: Colonel Canfield, I would like to ask you, the older literature is filled with reports, anecdotal and otherwise on the presence of pneumonic infiltrates after virtually any of the antimonials. Did you notice that in your series? It seemed to be very devoid of respiratory side effects.

COL. CANFIELD: My impression from reading the older literature is that this occurred more in the severely ill, the kala-azar patients, and they got a lot more complications from the drugs. They got the cough and the pneumonic infiltrate. They got cardiac toxicity. And some people then have gone on to say, well, they don't know what this means because a patient may have had a lot of other diseases, and we have not really seen that. We are, as I say, doing daily electrocardiograms, and we have seen maybe just a tad of T wave flattening, but really we are seeing nothing in the cardiorespiratory system.

DR. SIMPSON: I had one other question which may not be appropriate at this time. What is the status now in your laboratory of Allopurinol and the other conjugates? Dr. Joe Marr from Washington University in St. Louis read a very exciting paper at the Denver Meeting on the use of these

in vitro and in an animal model. I have forgotten which animal at the moment. It showed considerable promise as a drug which produced a static effect in vitro. You would wash the drug out, and the organisms would immediately regrow, begin to multiply again. I think he was dealing with promastigote cultures.

COL.CANFIELD: (Shrugs, no response.)

DR. BENENSON: Dr. Schultz?

DR. SCHULTZ: I think the most critical issue as relates to therapy of leishmaniasis in the military has yet to be raised, and I will raise it now, and that is the very pragmatic problem of supply of pentavalent antimony. It is an issue I raised to Dr. Beaver in 1972, when he was the Director of the Commission on Parasitic Diseases, and I raised it at that time because CDC was supplying Pentostam to both the civilian sector and the military sector, and we learned that the supply was to be cut off, and indeed, we ran short and were unable to supply it to some patients, including military patients, and the manufacturer, Wellcome Foundation was going to discontinue it, and I perceived this as a military and strategic type problem because as was brought out again at this meeting, leishmaniasis is in all of the tropics and could be in any potential theater of war, and yet our supply of Pentostam is so tenuous. It comes from one supplier who was about to discontinue it, and the

only alternative was Glucantime which is not a very good alternative.

DR. BENENSON: Let us attack both of those -- I am sorry. Were you finished?

DR. SCHULTZ: Yes.

DR. BENENSON: Brice, you have had some contact with Wellcome. Do you know if they are planning to cut off Pentostam?

DR. WALTON: Yes, this is a very real problem now, worldwide, not just for US military. Leishmaniasis is on the rise worldwide, and it is very difficult to get it. Pentostam, for years, was stocked by Wellcome from one manufacturing run. It is something like a 15-year supply was put down from one manufacturing run, and evidently the manufacture of all antimonial compounds is not a very exact science. There is an element of bats' wings and toads' toes involved, and the procedure is to make a run and then assay the product, and if does not meet the specifications reject it and start all over again, and evidently this is what happened with specia(?) with Glucantime. They had production problems for quite a time, and they just almost had the supply lines empty, and they got a fair production going, and the Arab nations with their petrodollars found themselves in the position to be able to treat the cases of tropica for the first time and I was told, and I cannot verify it, but

I was told that they bought up the world supply of French produced Glucantime which caused a severe shortage. None of the normal distributors throughout Latin America had any for almost a year and one-half. However, investigating this for PACO(?) I found that Glucantime is also produced under license by a Brazilian manufacturer and they have evidently had no problem, and it has been available at a much better price than the French produced, and as a matter of fact PACO now is negotiating purchase for several governments through Brazil. I understand, also, informal contacts with Wellcome, that they did or are shortly planning another production run. So, they are not going to run out.

So, there will be at least two sources in the Western world of pentavalent antimonials.

DR. SCHULTZ: Yes, there was some intercession with the Wellcome Foundation at the time that came up. They are continuing in the business of doing it, but it is still a very weak link in the chain, and I would hope the military would see fit to stockpile, presuming that it is stable, and I am not sure about its stability, or else get some American manufacturer to underwrite, an American manufacturer to make the stuff.

DR. BENENSON: Three year storage?

COL. CANFIELD: Three year shelf life.

DR. BENENSON: Is that an arbitrary shelf life or is

something of which there is actual degradation?

COL. CANFIELD: That is the manufacturer's shelf life.

DR. BENENSON: I know, but the manufacturer often -- I don't know if anybody is here from BOB, or FDA, I mean arbitrary dates are put on with no evidence of deterioration.

COL. CANFIELD: I know that the previous batch, the one that they are shipping from now, I believe, expires this year, and that is why they are coming up with this new big run. So, presumably the last batch they put together was three years ago, formulated.

DR. BENENSON: Do you know anything about its degradation?

SPEAKER: The labile on Glucantime has no expiration date.

DR. BENENSON: Since most of Latin America -- do you have any comments on the Brazilian glutamine?

DR. MARSDEN: No. I, of course, see it from the point of view just from the university setting and my use on the ward and in the field, and we have not had any holdup in supplies, but I cannot comment on it.

Of course, Pentostam is not available.

DR. BENENSON: Dr. Gunning?

CAPT. GUNNING: I wanted to ask Dr. Schultz, you have intimated that Glucantime was not a suitable alternative.

Do you mean to imply that because of their manufacturing problem it was not a suitable alternative? If the manufacturing were guaranteed and held stable, would it be in your estimate or CDC's estimate as good as Pentostam?

DR. SCHULTZ: It is a pentavalent antimonial. So, it could be used. We had problems physically getting it. That was one problem, then various problems of volume administration.

CAPT. GUNNING: But as near as anyone can tell here in this room there really is not too much difference in the therapeutic efficacy?

DR. BENENSON: No. Your question is one that I started to ask Colonel Canfield because he is the one who condemned it, and since it is in general use throughout Latin America, I think it is worth reviewing the reasons why you say that it is an unacceptable alternative.

COL. CANFIELD: It is unacceptable to us for two reasons. One, we couldn't get it, and we tried.

DR. BENENSON: All right. You know where to get it.

COL. CANFIELD: The second reason was that when we wrote to the company and we reviewed the literature trying to find sufficient preclinical toxicological data on which to base an IND we just could not find it. We could work the drug up, but we are talking about three or four years of work.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: I think the problem here is the very problem we face in any one of these international diseases that the information is difficult.

It was a French drug. It was put in South America by the French at a time when the French had -- Paris and Rio were very close. Everybody in Rio sent their children to the Sorbonne, and in consequence that literature is hidden and lost to us. I agree with you, it is not good literature. It is not good literature on Pentostam, but certainly the Pentostam there is more meat to it. You will never get it out of the Glucantime unless you are prepared to go around Brazil hunting lost files.

COL. CANFIELD: You are right. The literature on Pentostam is not very good either, and indeed when we sent our IND to the FDA they said they looked at how many patients we thought we might have to treat, and they said, well, because we had in essence submitted the same information that CDC had submitted, they said that that IND was okay for one or two patients a year, but if you are talking about more patients you need a lot more studies, animal studies, and so we are having to do some animal toxicology studies.

DR. BENENSON: Let me translate what you just said. Are you planning to do them or you would have to do them if you wanted to use it? You are not planning to do it?

COL. CANFIELD: We are doing it.

DR. BENENSON: Oh, you are doing it. Okay.

COL. CANFIELD: With Pentostam.

DR. BENENSON: Oh, with Pentostam, okay.

Let me pose a question, and maybe you all can think on it at lunch. You are talking about the military cost of this disease, the time lost from duty and so on, and I find myself wondering why we traditionally say that a patient with leishmaniasis must be hospitalized during the time he has an open lesion.

I think maybe we will leave that for you to think about, and I will raise that question after we come back from lunch.

Colonel Erickson, our traveling instructions again?

(Administrative announcement.)

(Thereupon, at 11:55 a.m., a recess was taken until 1:10 p.m., the same day.)

AFTERNOON SESSION 1:10 P.M.

DR. BENENSON: Before we go into the agenda item of considering the answers to the questions that Colonel Cutting posed, is there any more discussion on Colonel Canfield's protocol?

Dr. Marsden?

DR. MARSDEN: I would like to ask Colonel Canfield just a little more about this whole concept. It came as a complete surprise to me that there is no cumulative effect with pentavalent antimonials because the older physicians, and I am quoting now, Sidney Hamilton, Sir George Morrow, and these people. They all said that it was a cumulative drug, and you had to stop after 10 days. You are sure that in fact none is retained? It is all excreted, is it? The behavior of the pentavalents is different to the trivalents in this respect. Why are the trivalents held in the body and the pentavalents not?

COL. CANFIELD: I cannot say that there is no tissue retention, but by and large the majority of the material is excreted within 24 hours. There could be some accumulation, let us say in the heart or there could be accumulation in the macrophages. In terms of the total dose that you deliver it would not be measurable. In other words, the way you calculate this if the elmination half time is four hours, that means that there are six elimination half lives

within a 24-hour period of time, and so if you just start out with 100 percent, 50 percent, 25 percent, 12-1/2, you get down to about 1 percent of the drug that is left over after 24 hours. So, there is a little bit of drug, and it could conceivably be in a tissue in which it could either be toxic or therapeutic, but in terms of the vast majority of the drug it is certainly all gone.

DR. MARSDEN: What about this business of the trivalent, there is a difference in handling?

COL. CANFIELD: Yes.

DR. MARSDEN: Because trivalents are terribly toxic. I don't use them anymore, but I had a lot of experience in the old days with trivalents, and the pentavalent in comparison are almost magical.

COL. CANFIELD: Yes. In metabolism studies with the two drugs, again, pentavalents are almost all excreted in the urine, and the trivalents, I believe come out primarily in the feces, as I recall. They are either excreted in the bile -- in other words, they are recovered in the feces, and they are metabolized more by the liver.

In terms of recovery of dose delivered I believe, yes, there is more tissue retention of the trivalent, although the kind of study that I just showed you with Pentostam I do not recall a similar study with a trivalent.

DR. MARSDEN: But the study you showed us by Otto

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was not actually Pentostam, was it?

COL. CANFIELD: No, it was Dibanose(?), a pentavalent --

DR. MARSDEN: Which is different again.

COL. CANFIELD: But it was a pentavalent antimonial, and in terms of, there is another metabolism study in animals which showed that the pentavalent antimonials are handled quite similarly.

DR. MARSDEN: It has a practical point because for instance, what we are doing in Brazilia, we are using Glucantime in three series of 10 days, a fairly standard thing as set down, but if what you say is true, we could shorten our hospital stay that we have to bring a lot of them into the hospital. We could shorten our period of hospital stay and save money, if we gave them the whole three right off the bat, as it were, giving 1 gram of drug per kilo per series over 10 days and having to bring them in after a fortnight for repeat and yet again to repeat that. It is costing us a lot of money.

I mean would you advocate that we --

COL. CANFIELD: That is the kind of question that can be studied,but it hasn't been studied. In other words, you can do tissue accumulation studies. You can do this in animals, and you can determine whether or not the drug is accumulating in certain tissues over a prolonged period of

time, but if the tissue -- if the whole body elimination half time is four hours or less, why you see virtually no accumulation, but again I showed you plasma disappearance curve which in this case represents the majority of the compound, but you also need to look at target tissues.

No, I could not advocate it without some very sophisticated studies.

DR. BENENSON: Dr. Gunning?

CAPT. GUNNING: Several of us have had a feeling in the past that, Dr. Walton and I, that perhaps 20 days of treatment with a reduced dosage, a longer span of treatment might be influential. I don't know if in the experience of anyone here -- has anyone ever laid that to rest? That would be point number one.

The second point is that I guess I could best tell this by an apocryphal story, a retrospective story. The first case I saw in California on my return of a marine who had been in Panama was just at the time when the Flagyl papers made their eruption, and naturally we decided well, perhaps we had better try Flagyl. Well, the point I am making is that on culturing this man who was only two to three months into his illness I finally got to the point where I got the negative cultures, certainly just before I tried the Flagyl. Otherwise, it would have been listed as a Flagyl success, so that I am not sure that the Pentostam can be blamed for the

cure, nor am I sure if you are going to have failures that the Pentostam is the reason for the failure, and I realize the design of your study is difficult at best, and then the third part of the question is are there other therapies that anybody is considering, such as combination therapies, Amphotericin to break it up and put something else in, a la fungus therapies where we are using a combination of Amphotericin and 5-FC? Has Rifampin been tried, combinations Amphotericin and tetracycline and things of that nature? Is there any experience with that? I know of none.

DR. BENENSON: Dr. Neva?

DR. NEVA: I believe Rifampin has been tried, but again only in just a few cases. The trouble is I think you cannot generalize with the response to treatment number one of visceral leishmaniasis and not just then all of the cutaneous leishmaniases I think will depend upon varieties of organisms, whether you are dealing with tropica major, whether you are dealing with the braziliensis panamensis or mexicana. I think these may give you some differences in response.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: I would like to come in on that. I agree with that, Frank, but it is even more complicated because we have no way, really, of evaluating the literature. Still they are getting one case in, you know, into the

literature as a treatment success or failure, because there is no way, really of standardizing how these cases are evaluated as yet, and it does not just depend, in fact, which is extremely important, as Frank says, on what type of leishmaniasis is present in the area but also what is, for instance, the incidence of spontaneous healing in the area. Spontaneous healing undoubtedly occurs. It is very difficult to get a gauge of this. It probably can best be done by a retrospective study of people with scars who have not sought specific treatment, because you can hardly leave the people and not give them anything, but if you don't know this in the evaluation of what happens to your patients it becomes very difficult on the drug because they could have done it anyway.

Another factor is what criteria are you going to use for the evaluation of success with the drug, and our feeling where we have got this terribly difficult form is that we would like to use four criteria, not only parasitological cure but histological cure, immunological cure as well. We would like to have these. I have, also, got another one which I have lost in the course of my discourse, but I will find it. We have actually got four. Oh, yes, it is clinical.

So, we feel that we would like information on all these four, really, and also over what degree of time are we

going to standardize this data? I quoted a case 20 months afterwards, relapse in the nodes. So, obviously, the studies like studies from Panama, like Brice's paper on provolume(?) a very important factor was he gave us the length of time the patient had been followed up. A lot of these papers never tell us how long they have been followed up.

DR. BENENSON: Dr. Neva?

DR. NEVA: Maybe I am coming to your defense, Craig. After being somewhat critical of this experimental design, I think actually the design that has been outlined to us probably is about as good a thing as you can do to try to get these answers.

In otherwords, if you systematically treat according to various schedules and then you culture the lesions, looking for persistence of organisms, I think this will at least tell you on a comparative basis with each regimen of treatment how easy or difficult it is to recover organisms from the lesions. Now, that is at least one bit of data that may not give you the total answer, but I think it is going to be useful in interpreting this, and then clearly you will have clinical response to go on too. If anything the way I would maybe advocate thinking about if you are going to make any modifications, I would leave perhaps a little bit longer time interval if you can, just to be realistic in carrying this out, a little bit longer time

interval between the shifting to a different mode of therapy, leaving a couple of weeks, at least, from the time of ending one regimen, doing your biopsy or whatever method of culture you have and then shifting them over to another.

SPEAKER: For toxicological --

DR. BENENSON: Canfield?

DR. NEVA: No, to allow time for several possibilities, that you may inhibit organisms and make it difficult to culture them for a few days or maybe a week and just give time for being able to recover them again.

COL. CANFIELD: Are you saying that you would not treat as soon as you got a positive culture?

DR. NEVA: No, I would go ahead with your protocol as you have it because otherwise you don't have the same way of handling each one of the treatment schedules. I think you have to do the same with each one.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: The maximum period of time that could elapse between treatments would be 34 days. That is with the culture, last culture taken 13 days after treatment and keeping the culture for 21 days before we consider them negative. If we do not discover it until the 21st day that it was negative, it would be a 34-day lapse between the last course of therapy and the next course if it became positive the 21st day.

DR. NEVA: I think probably a more important issue is the duration of time after completion of one course of therapy and the time to do your culture. As I recall that was two days for the first one, three days, and then 13 days. Perhaps that is reasonable.

MAJ. HENDRICKS: On Dr. Marsden's point about follow-up, it is pretty difficult because there is a fairly rapid turnover, and Dr. Takafuji and I have been discussing this quite a bit of how far we can follow this particular battalion and still have some assurance that we are going to get 80 percent of them, 90 percent of them, whatever. There is a fairly rapid turnover in many of these airborne outfits of personnel being dispersed to other areas in the United States or getting out of the service, and if you let it go too long you just won't be able to get them back to follow them up.

Once they are out of the Army it is very difficult to get them back and follow them up on a protocol,that is an Army protocol.

DR. MARSDEN: Of course, it is not anything like we have to face with a wandering Brazilian population out there in the bush. So, if you chaps don't do it, I don't think we will ever get very really satisfactory follow-up, even though all our cases are much more severe. They just get lost.

COL. CANFIELD: We are establishing a framework from which a follow-up study can be constructed, that is we are getting as much information about the people, what their permanent addresses are,who their relatives are and such that even though they do get out of the service we can at least do a questionnaire follow-up at some time in the future.

DR. MARSDEN: If I could just come in there again, just to retell our small experience still, only two and one-half years in Brazilia, I mean we have found and it has been reported by Walton and others, that that fluorescent antibody test is very useful in assessing follow-up, and it does drop in the few places that we have been able to study, and it helps us in assessing really whether we have cured the patient.

Another tack that we are pursuing is we are doing a collaborative study with Ridley who is the expert on the leprosy granuloma in the skin, and of course has changed the management of leprosy by his interpretation of how the granuloma changes as therapy progresses in leprosy, and it struck us that possibly he could help us in leishmaniasis which has many similarities to leprosy if you could devise some sort of histological formula he could apply to leishmaniasis, and he has a paper in draft on this. He has not been as successful as he has been in leprosy, but some progress has been made in interpreting what is happening

to the granuloma and what this means in terms of the possibility of a relapse.

COL. CANFIELD: Just to respond, a couple of times you mentioned serology. We are, of course, drawing sera, and if Carter can ever develop a test that will work --

DR. BENENSON: Let me raise the question that I raised before the break, and that is the development of protocols which require hospitalization. Is there really a reason to hospitalize the patient who has a cutaneous lesion that is not very extensive and so on and so forth?

COL.RUSSELL: Only if you want to treat them.

DR. BENENSON: But you can treat them on an outpatient basis.

COL. RUSSELL: Not under the protocol.

DR. BENENSON: No, no argument on this protocol. No, I am talking about you are evaluating therapeutic regimens, only one of which could be used on an outpatient basis. That is protocol A, because they could come in daily for their intravenous dose, but I am raising the question.

I mean in the earlier discussion of Dr. Takafuji's presentation he had a note that this represented so many hundred man days lost, because I think we have always handled leishmaniasis as a diagnosis that requires hospitalization. We put all military personnel in the hospital the moment we make the diagnosis. I raise the

question is that necessary?

Captain Gunning?

CAPT. GUNNING: I have had experience with treating two patients as outpatients in San Diego. These were Army Reservists who contracted their disease in Panama, but they were going to school and working and just could not get off.

DR. BENENSON: They were not military personnel?

CAPT. GUNNING: They were not military personnel.

DR. BENENSON: They are harder to maintain and get hold of.

CAPT. GUNNING: And in order to treat them it was either a question of doing it this way or almost not doing it at all. Those chaps stayed in school. One of them drove a cab all night, and they had really no untoward effects. We monitored the laboratory tests. The only thing I insisted on was that they remain in the clinic for a period of two hours following their intramuscular injection, and we did not have any problem.

Now, as an analogy to this, we do this in Amphotericin intravenous therapy in coccidiomycosis in California --

DR. BENENSON: That is a much more toxic drug.

CAPT. GUNNING: It is much more toxic, and we do it all the time as outpatients. You just keep them there until they have had their infusion and then let them go.

DR. BENENSON: That is why I raised the question, because in Kentucky we gave Amphotericin as an outpatient drug,much more toxic than the pentavalents we are talking about, but, all right, let us assume that I have cast a germ of thought into your minds and let us go ahead.

Unless there is more on the protocol that is underway --

Yes, Dr. Simpson?

DR. SIMPSON: I would like to introduce one other question which you might want to come back to later. Obviously there are probably better means of prevention than chemotherapy, but I wonder if any consideration is being given to chemoprophylaxis? Obviously in Dr. Marsden's setting this would be of little or no value, but in a military operation where you are dealing with two, two and one-half weeks of acute exposure this line of approach if one could get a residual depot drug which was safe and effective in that role, perhaps it would be worth looking into.

DR. BENENSON: Dr. Canfield, what is the hope?

COL. CANFIELD: It is much easier if you have got an effective therapeutic drug and you know how much drug is required. Then you are right, it becomes a technical problem on how to develop a sustained release formulation. I don't think we have gotten through the first step.

DR. BENENSON: Dr. Neva?

DR. NEVA: I was going to second the suggestion about at least thinking about the possibility of testing the prophylactic drug. One of the nice things about your experience is that you have battalions going in, and you have already a baseline. You know pretty well what to expect. I think you could get a reasonable idea of whether a prophylactic drug is going to give you at least a dramatic response, and if you want a suggestion for a candidate, I would suggest pentamidine.

DR. WALTON: Why and how and how often?

DR. NEVA: One dose, because it is effective in the treatment of visceral leishmaniasis, at least some of the cases that are resistant to antimonial drugs because it has some value in the treatment of other protozoan infections, prophylaxis, African trichosomiasis. At least think about it as a possibility. You might even test it in experimental animals first.

DR. BENENSON: Dr. Walton?

DR. WALTON: I would like to register a minority opinion on two points. One, it is true pentamidine has been reported to be effective in some cases that are resistent to antimonials, but when it is used as a primary drug the efficacy rate is not as good as antimonials.

DR. BENENSON: In established lesions?

DR. WALTON: Yes, and the whole idea of prophylaxis, I think is a little bit shakey if we are thinking about areas where braziliensis occurs with the danger of delayed development of mucocutaneous lesions.

We obviously don't have any good criteria with which to judge a cure. So, we cannot really just how well we are doing with the prophylaxis. What we might be doing is giving incomplete treatment and therefore setting your patient up for these delayed onsets. Until we have a way of detecting infections, I think it would be pretty shakey in the braziliensis country to use a prophylaxis.

DR. BENENSON: Dr. Farah?

DR. FARAH: I accept all these objections, Dr. Walton. However, in thinking about prophylaxis, maybe one can think of certain drugs like cycloguanil pamoate, a Camolar which when used in the treatment of both cutaneous leishmaniasis of the Old World and also of the New World seems to act somewhere about two months after a single injection is given. This may be the type of drug that could be considered at least initially in a trial period.

DR. BENENSON: Maj. Hendricks?

MAJ. HENDRICKS: We have been working with an animal model for quite a while that Dr. Walton and I worked with in Panama. It was the African white tailed rat, mystavis zabocoidadis(?) And we thought about therapeutic

or chemical prophylactic trials. We have run treating the animal at the same time that we inoculated him and prior to infection with a 10-day regimen which would be equivalent to the dosage that the humans receive. What it did, in effect was prevent the development of the lesion for seven weeks. It did not prevent the lesion from developing. It just retarded it for seven weeks. So, it goes along fairly well with what Col. Walton said, with Glucantime.

DR. SCHULTZ: In our series where we used it for pneumonia, admittedly a longer course, but we are still putting pentaminine on board. It was 40 percent toxicity of moderately severe to severe toxicity.

DR. BENENSON: How many diabetics?

DR. SCHULTZ: You mean did it cause?

DR. BENENSON: Yes.

DR. SCHULTZ: There were some. I don't recall the percentage.

DR. BENENSON: Dr. Simpson?

DR. SIMPSON: I would like to ask Colonel Walton a question of information primarily. Was there anything odd about the recurrent cases of cutaneous leishmaniasis that appeared following the dose of Camolar that was given during your 1965-67 study?

Were these worse or better or just simply the same?

DR. WALTON: No, I don't think there was any real difference. We probably did not have the experience when we were doing the Camolar that we developed later, but I think there were probably more out and out treatment failures with Camolar, that is no apparent effect, very little healing effect, and in a way it might be better because we had, I think we encountered a higher rate of recurrence after say three months with pentavalent antimonials. As a matter of fact, some of the statistics that were developed we never saw the patient when he had his recurrence.

We used several devices to try and be notified if there was a recurrence, and many of these treatment failures were sent away from Gorgas Hospital in the Canal Zone and recorded as treatment success. Six, seven months later, I don't know what the longest period was, but we would get a postcard notification that we put in the medical records back from another military hospital and two cases from Veterans Administration Medical Facility saying that the patient was there with a lesion, and he has a history of having had cutaneous leishmaniasis. So, I think that answers the question.

DR. BENENSON: Anything else?

Let us move on to the questions that the Board is being asked to answer.

I took the liberty of asking Carl Johnson who has

been treating leishmaniasis or cutaneous leishmaniasis since 1930 for his responses to these four questions, and they are being distributed to you.

We will take them up one at a time. So, don't read on ahead. They represent on the whole the opinion of one man who has probably seen more leishmaniasis in his life span, with all due deference to Brazil than all of us put together. Essentially he is describing the methods that are used at Gorgas Memorial Laboratory.

All right. Now, our first question is what is the most appropriate therapeutic regimen for individuals developing disease? Now, this is clinical disease with clinical manifestations. Of course, that in essence is what we have been discussing for the past hour, and where Carl Johnson recommends or is convinced pentavalent antimonies are preferable, he has been using Glucantime. We have not been using Pentostam in Panama. We have heard the reasons why, at least for the time being among US personnel, I presume you cannot use glutamine. Why do I keep calling it a carbohydrate? Glucantime. Is there any further discussion on that? I mean those of us who are south of the border apparently are all using Glucantime.

DR. MARSDEN: For the same reason as those north of the border are using Pentostam. We cannot get the other one.

It is not that we have any particular preference.

DR. BENENSON: And there are no comparative studies that I know of that says one is better than the other or worse than the other.

All right.

The next thing that he alludes to is the use of Camolar, and he points out that when it is used improvement does not become manifest. Now, he is talking and Carl rates the treatment of cutaneous leishmaniasis in terms of clinical aspects. If the lesion heals the patient is cured. I think I implied earlier today he still has possibly leishmanial infection, but he doesn't have leishmanial disease, and he points out that with Camolar the improvement of the lesion becomes manifest after one month and healing is not completed before two or three months. It is much slower than the pentavalent antimonials.

Any discussion on that?

DR. MARSDEN: Well, its thorny, this Camolar. I don't like to get into it because I have never used it myself, but I am confused. I mean here in our area I think we go straight on to Amphotericin B because we have such damaging braziliensis type disease that we cannot afford in Central Brazil to wait that long.

Another aspect that raises, that comment is something that has to be said in comment that while I entirely

agree this represents unusual experience and Dr. Johnson has more experience than anybody else, we must remember it is experience based on Panama, and unfortunately we don't have a Peruvian with us today, and we don't have various other representatives of types of leishmaniasis, those people who might give a different opinion again.

DR. BENENSON: One reason that we have on this Ad Hoc Committee today someone from Brazil and someone with Middle East experience is because the military has to consider the therapy of cutaneous leishmaniasis from a global perspective and not purely from the point of view of some soldiers at Ft. Bragg that have cutaneous Panamanian infection, so that I think we have to look for the broadest globally most effective therapy. That is reinforcing your arguments.

DR. MARSDEN: Yes. If I could just comment again, I mean our indication for using Amphotericin which for us with our small facility is not something we like to use because it does mean in our case hospitalization, an intolerant patient a long protracted course of therapy, is someone who has not responded to our full course of Glucantime and who has severe mucosal damage. We are not using it, in fact, in our circumstances for treatment of cutaneous lesions, because we have such a call on our resources, but that is purely a practical point.

DR. NEVA: Would you agree on the dose that he recommends if you do have to use Amphotericin, 1.5 to 2 grams total?

DR. MARSDEN: Yes. I think that it is fortunate that, in fact, lower doses than those used in deep systemic mycoses appear to be very effective in severe mucocutaneous leishmaniasis. I could show slides. I won't. There is not time, but I could show slides of people who have failed after such a course. There is always the patient who does not -- but this is the sort of course which Sampeon who introduced it into Brazil, the sort of courses that he found were virtually uniformly successful.

DR. BENENSON: Again, looking at it from the point of view of curing the manifest lesion, Carl is very happy with the application of local heat which, as he says here, in some instances has produced beautiful results when drugs were contraindicated and so on.

Again, I stress that I am sure that the infection persists in these, but the lesion is cured, is healed. I think that is the better term.

All right. Anything else on therapy? This is as much as Carl has. He excised his own lesion, and he points that out here, and of course when I challenged him he says, yes, he still has a good high serological titer even though he did that excision 20 years ago or something like that.

Again, he cured the lesion by excising it, but infection with the parasite, I am sure, persists within the body.

Dr. Farah?

DR. FARAH: In discussion of the treatment of cutaneous leishmaniasis, I think one should be very careful in defining exactly what parasite one is dealing with. For instance, our experience with the treatment of Leishmania tropica is first we don't require hospitalization for the treatment of a cutaneous lesion of Leishmania tropica.

Most patients with Leishmania tropica have a single lesion or if they have multiple lesions, the multiple lesions are the result of multiple bites rather than the result of dissemination.

I exempt from this, of course, the diffuse cutaneous leishmaniasis which is another story. So, we don't really feel that there is systematization in infections from Leishmania tropica as such.

Secondly, local treatment, not necessarily excision but if you have a single lesion which is very recent, obviously incision has worked, and we have not been able to see patients -- we have not seen patients who have relapsed afterward, but even local treatment with cryotherapy, like with the use of carbon dioxides now or even liquid nitrogen has been very successful. It is very simple to use. It can,

of course, be available in the field. The treatment could result in well determined scars rather than left to the scar of the infection itself. So, consequently one gets better cosmetic results and of course very effective therapy.

Even patients who have a recidivans infection or a chronic leishmaniasis, and these are rare cases in which there is a prolonged infection as in the chronic Leishmania, for instance which might last even for 15 or 20 years, the treatment with local cryotherapy could be very, very effective.

Thirdly, our experience with Camolar agrees with the experience mentioned here. We have, also, about a rate of 80 percent improvement beginning two months after a single injection, but this is limited again to the treatment of acute cutaneous leishmaniasis and by acute cutaneous leishmaniasis we imply that lesion that heals spontaneously within a year.

We have not had any success with Amphotericin B in Leishmania tropica. We have used it just on an investigational basis, and we don't really feel the justification of using even the antimonials or the Amphotericin B which are in themselves more toxic and produce more morbidity than the actual lesion of Leishmania tropica, cutaneous leishmaniasis lesion.

DR.WALTON: It has always bothered me that the last resort of treating military patients with leishmaniasis

in Panama has been excision of the lesion. In the series that were treated at Gorgas Hospital there were quite a few which at the end of the CDC protocol still had lesions in which you could culture organisms. The lesion was excised. It solved the problem, and the patient was sent on his way.

To me this is the best indicator of the effect of chemotherapy. You have got your parasites localized in one spot. You cannot kill them with drug therapy. To excise the lesion at the time you give the course of therapy, I think is defeating your purpose. You are doing away with the best indicator of therapy that you have. I think one of the recommendations that we ought to make in an area where there is risk of braziliensis infection is that the excision not be used.

DR. BENENSON: I am not clear on the point you are making, Brice. You say that if you give drug you are eliminating your best criterion if you are excising --

DR. WALTON: Yes. If you have parasites at the site of your initial lesion and they persist after treatment, it is a very good indication that your treatment has not been adequate. However, if you excise those lesions at the time you give your drug I see no reason for the belief that some of these physicians apparently have that the parasites in another location will be eliminated if they are not eliminated from the primary ulcer.

DR. BENENSON: Oh, in other words, if the particular parasites are refractory to the drug you are not achieving anything at other sites by giving the drug, and you might as well cut it out and if there is anything else worry about it later if it shows.

DR. WALTON: I am not convinced that this is justified. We are looking in Panama at the apparent incidence of mucocutaneous involvement and it is very low, and whether this is due to having two types of Leishmania which are infecting people and only the rarer type causing mucocutaneous lesions or whether it is a variable host response I don't know, but it is very obvious that there is much less, much lower percentage of people infected with leishmaniasis in Panama who develop secondary espundia than there is in Brazil or anywhere down the east side of the Andes.

So, we are fortunate that we are dealing with a parasite where this danger is minimal, but I think it would be very dangerous to develop policies based on the experience in Panama when we might have people exposed in other areas. Therefore this would be the worst possible measure you could take in terms of judging whether or not the patient is adequately treated.

MAJ. HENDRICKS: One patient we have identified puts together most of these doctrines into one patient. He

has now been infected for over 20 months. His lesions were treated twice with x-ray, resolved themselves temporarily, reappeared somewhere in the initial area of the original lesion, received another dose of x-ray, and they resolved temporarily and then reappeared, received a course of Flagyl, unsuccessful, surgically excised the lesion. It appeared a slight distance away again and then received a course of Pentostam, was found culture positive and now received a second course of Pentostam and has been culture negative for over a month now. That is almost the entire gauntlet of possibilities.

DR. BENENSON: I did not hear the baseball bat involved there. I think the lesion should have been hit once or twice and see what happens.

Any other -- Dr. Marsden?

DR. MARSDEN: To just raise this typical question in your protocol, of course, you are basing your subsequent treatments with Pentostam on the results of your cultures. Now, the doctrine, of course, is that you give the three series because nobody was doing cultures like this before, and of course, yours is an investigational trial, but I mean I am always bothered about the possibility to put it very simply and rather interestingly possibly, but the first course knocks down the parasites by 80 percent of the population and the next course gets, say, 15 percent and

the last course eradicates the other 5. If it is a situation like that, of course, and the cultures -- and you don't know what the degree of sensitivity of the cultures are at this point, you could, in fact, end up with a group of patients that are partially treated, as it were, but go on years later to develop problems, and you difficulty is to decide whether you are going to do the three courses all at once or you are going to do this sort of thing -- fortunately, it is your problem, not mine.

DR. BENENSON: But you have to help solve it. That is what you are here for.

Dr. Gunning?

CAPT. GUNNING: This is a terribly difficult thing to talk about because we have so much baseline information that is lacking. For instance, I am willing to make a few givens, and I am not sure whether I should be doing that or not, that the spontaneous incidence of mucocutaneous leishmaniasis in Panama is less than it is in Brazilia, and the minute I have said that, I really don't know if I have said anything correct or not.

The real problem that we really cannot come to grips with in experimental design for the practical purposes of the problem at hand is that we are dealing with strain differences. We are dealing with host differences. We are dealing with, for instance, observations in animal models

that the Leishmania of a mouse will survive in the macrophage of a rabbit, whereas the Leishmania of the rabbit will survive in the macrophage of the mouse and not vice versa. What we are really dealing with is a macrophage problem.

Now, what is Pentostam doing? Is Pentostam turning on our macrophages so that we can kill the leishmania? Does it do so imperfectly on the first course and then perhaps gets a little bit of boost on the second course or whatever other drug we throw in, and I don't know how to evaluate that, because really I will come back to my major premise, I think this experiment that Dr. Canfield is going to do will be a howling success if he has 100 percent eradication, but anything short of that is really not going to yield any great answer.

DR. BENENSON: Any other comments?

Dr. Farah?

DR. FARAH: I appreciate very much bringing in the macrophage into the picture of leishmaniasis because I am one of those who completely agree with the fact that it is not only the species of the parasite but it is certainly the response of the host, and there is a lot of evidence I think now to show that the type of the species of the host determines the outcome of the infection with Leishmania.

For instance, if we take Leishmania tropica and in fact, CBA in mice for instance in a certain dose, let us

say two million parasites you would get within three weeks a definite infection at the site of the inoculation, and that would last for about three or four weeks, and it would heal just like the regular cutaneous leishmaniasis produced by Leishmania tropica. However, if you inject from the same culture, the same number of organisms into the above C(?) mice, you will get an unhealing lesion that ultimately will metastasize. In fact, the mice, if you inject them at the base of the tail will ultimately even lose their tail because of the severity of the infection, and you have a complete non-healing ulcer that is produced with evidence of metastasis, and I think there is a lot of evidence now that is appearing with the use of macrophages and macrophage cultures and studying the relationship between the particular parasites and the different macrophages that show that there is a peculiar adaptation between a particular species of the organism and the particular host, for instance, the presence of certain receptors, the non-killing of the parasite in certain species, the parasite possibility of multiplication within a particular species of macrophages.

I think at the present with the availability of macrophage cultures and possibility of infecting macrophages with the various species of parasites it may be possible that one can study the question of response to the parasite, of the parasite to the drug or the response of the macrophage

to the drug and what is actually happening could be maybe worked out in this system.

DR. BENENSON: All right. Let me read you something which would be the beginning toward a consolidation of an hour's discussion.

In answer to Colonel Cutting's question we might respond thusly: Pentavalent antimonials are presently the most appropriate therapeutic regimen for individuals developing disease. Amphotericin B is a valuable drug, especially for mucocutaneous disease. The differences in leishmanial strains requires that the design of therapy must depend on the characteristics of the disease as seen in the locale where the infection is acquired, for example, in certain areas simple excision or cryotherapy constitute adequate therapy which in other areas would constitute dangerously inadequate therapy.

DR. MARSDEN: It does not sound very helpful really.

DR. BENENSON: I have to ask if that is or is not helpful. In other words, I think that is the sense of what I have been hearing, that it depends on where you got the infection. If you got it in Brazilia you will not handle it they way you will if you got it in Costa Rica or if you got it in the Middle East. If you got it in Panama you are not going to handle it very differently than you would if you got it in Costa Rica, for example, going into local geography.

Dr. Gunning?

CAPT. GUNNING: Dr. Benenson, I would like to say from a pragmatic point of view I am happy with that statement.

DR. MARSDEN: I would like, for instance, that bit about Amphotericin, I think it should be said that if adequate pentavalent antimonials have not been successful in the case of mucosal disease Amphotericin --

DR. BENENSON: That is what I had originally when I started to write.

DR. MARSDEN: Yes. The other thing --

DR. BENENSON: If pentavalent antimonials fail, Amphotericin B is a valuable drug, especially for muco-cutaneous.

DR. MARSDEN: Yes.

DR. SIMPSON: Dr. Benenson, I would like to ask a question, again, out of sheer innocence, I think, of Dr. Marsden. Considering the pharmacologic effects of Amphotericin, a point that Dr. Gunning alluded to earlier, would there be any rationale in using this drug concurrently with the final or third course of Pentostam?

If you have had two courses of Pentostam that have obviously failed with culture proof of that why not give concurrent --

DR. MARSDEN: The problem is, you see, as I said, in our form of leishmaniasis in the area we just don't have

the success. I think we would have more success, say, if you were doing the cultures, but I am sure it would not reach what you are getting in your people from Panama. It is difficult ground, and therefore the clinical evaluation is important. These people improve gradually, so that if you are giving three courses of Glucantime, a 10-day course and then a fortnight's rest, often by the time you reach the third course, still they are only just beginning to improve, and they go on improving after the third course. So, it would not be justifiable to combine the third course of Pentostam.

I think you have got to wait and see whether they are going to respond to the pentavalent antimonial which brings me to another possible modification which is, you know, it rather worried me when it was said here from the Chair that it really is my responsibility to try to work out something for this study. It is not that I can, but obviously your situation is different to mine, and it might be perfectly logical and justifiable for you to give one 10-day course to your people in Panama, because the great majority probably will heal, and it will never come back.

It would not be justifiable, I want you to know, to do that in Central Brazil where I think three courses should be given.

So, the point is once you get into splitting it up into the areas of the world it becomes a very long recommendation,

but your comments are entirely different again, aren't they? I mean you would not dream of using Amphotericin or antimony.

DR. FARAH: Except if somebody has really many lesions, otherwise no.

DR. BENENSON: Dr. Gunning?

CAPT. GUNNING: Dr. Marsden, are you saying that for your Brazilian cutaneous leishmaniasis you are giving three courses of antimony back to back regardless of whether they are mucocutaneous lesions or not?

DR. MARSDEN: Yes, we have got so much mucocutaneous disease, yes, we are doing that.

CAPT. GUNNING: Wow.

DR. BENENSON: That, of course, is the sense that I have tried to get in here. I don't see how we can this afternoon give a geographic glossary of the ideal therapy in every community, but it seems to me that if troops are going into an area it becomes the responsibility to establish what is known in the literature of that country with regard to leishmanial therapy.

Dr. Walton?

DR. WALTON: I think it might be appropriate now to mention, and it might be begging the question a little bit, but the TB MED(?) on leishmaniasis that the Army had, and I believe it is dated 1947, and I would venture to say that 90 percent of the military physicians who have a case of

leishmaniasis never think about looking for a TB MED for guidance. So, this very useful tool for guidance is just not available right now in the Army. So, possibly a secondary thought might be to suggest that someone bring this information together in the form of a TB MED. I can say this now. I was afraid to mention it for many years because I knew what would happen.

CAPT. BROWNLOW: That TB MED was '45 and revised in '47, General Eisenhower, Chief of Staff of the Army signed it, but the entire thing is concerned with visceral leishmaniasis. It only casually mentioned cutaneous and does not offer any therapeutic or very little information whatsoever.

DR. WALTON: It is based on North Africa-Mediterranean experience.

DR. BENENSON: I don't think that is an inappropriate recommendation for us to make to the services.

CAPT. GUNNING: Dr. Benenson, I mentioned to Dr. Simpson on the way over here this morning that I was at the USC library Friday looking for a World Health Organization TECRUP(?) series, and there is not anything in the last 10 years on cutaneous leishmaniasis from that source.

DR. BENENSON: Shall I add, and remember I am merely trying to write what the Committee wants me to write.

I am just the synthesizer.

Dr. Schultz?

DR. SCHULTZ: Well, you mentioned WHO and it made me think of their new tropical disease research training program, and one of the six diseases is leishmaniasis, and I presume that they do have the technical group on this that is operative and making recommendations, but I don't know who the individuals are.

DR. WALTON: There are two members of the WHO scientific working group here in this Panel.

DR. BENENSON: It is recommended that the TB MED -- do you know the number? I don't know why 47 came to mind. That is probably something I -- well, the TB MED on leismaniasis be brought up to date to include appropriate information. Is that vague enough?

Is this then the sense of the Committee? I will read it again. Pentavalent antimonials are presently the most appropriate therapeutic regimen for individuals developing disease. If these fail, Amphotericin B is a valuable drug, especially for mucocutaneous disease.

The differences in leishmanial strains require that the design of therapy must depend on the characteristics of the disease as seen in the locale where infection is acquired.

For example, in certain areas simple excision or

cryotherapy constitute adequate therapy which in other areas would be dangerously inadequate. It is recommended that the TB MED on leishmaniasis be brought up to date to include appropriate information.

Dr. Walton?

DR. WALTON: I would prefer to add, say, that pentavalent antimonials is the most appropriate therapy but it is not completely acceptable or effective, and there is a need for improved chemotherapy. I think this might give someone some maneuver room to get support to continue work for new agents.

DR. BENENSON: I tried to imply that by saying, "Is presently the most." In other words, I was hoping the word "presently" indicated that we don't think it is the ideal but as of now it is the best we have.

Any other points? Are you satisfied with my emphasis on presently.

DR. WALTON: Yes.

DR. SIMPSON: Would it be appropriate to include a negative comment on the drugs that may possibly be used which are still available and which have had some fluorish in popularity, at least in the literature? I am thinking particularly of metronitazol(?) in a negative way that these are not effective and should not be used?

MAJ. HENDRICKS: Still regardless of the studies

that have been published you still get individual cases reporting success with metronitazol or whatever here in the United States. You get this fairly frequently, one or two cases.

DR. BENENSON: What I am busy cogitating is if we name something else will somebody read that and say, "Hey, metronitazol is something they listed as a therapy,"and they will miss the negative. This is what I am hesitating on.

DR. MARSDEN: Because there is no organized drug program on leishmaniasis when any drug for parasitic disease comes out it gets tried, and of course, it might be useful as has been pointed out to mention the recent drugs of parasitic disease which almost have a vogue. They are always tried in all sorts of things, aren't they? So, it might be worth while taking those ultimate ones. For instance, we have been working recently on lantig(?) which got favorable reports. It is useless.

It is not quite useless, but it is no good in comparison to this.

So, if we had, say, Flagyl, lantig, and Rifampicin was raised here, useless, tried in the Andes and it does not work.

DR. BENENSON: What about a generic statement that while many other drugs have been tried, no value has been shown. Now, that does not entirely fit Camolar, but

I hear none of you today saying that that is the drug that should be put in use.

The inadequacy in this therapy I tried to imply a while ago on the hospitalization. We need certainly in the non-military world an oral drug that can be given to the person who works out in the jungle where he gets the infection. We haul them into the city for at least a two-week period. He loses his pay. He has to pay for living there. It is a great financial, economic crisis for the person who gets leishmaniasis.

All right. I will add "Many drugs have been reported effective on inadequate trial." How is that? They have failed adequate trial. They have failed when properly tested, and then we are not naming anything.

All right. Are we all happy with this?

Not hearing any objection we will proceed to the -- yes, all right?

DR. MARSDEN: It is just the ultimate sentence, whether you should actually put in that they have failed when they have been properly tested because some of these have never been properly tested.

DR. BENENSON: I know that.

DR. MARSDEN: I don't think there has been a proper test of Flagyl. It depends on what you mean by proper, what criteria you adopt. You could get someone writing

in and saying, "What was the proper trial?"

DR. BENENSON: Fair enough, but I began to get worried when I had found myself having written, "Many drugs have been reported to be effective on inadequate trial." This might say, "Let us go ahead and try it." It has been found to be effective, been reported to be effective. That is why I -- really, I have many drugs of questionable value have been reported to be effective on inadequate trial. Somebody British should be writing this. They are the people who handle the language.

DR. FARAH: Why not say that they have been found effective in initial trials but not that many maintained this on subsequent long-term use or something like that?

DR. BENENSON: Why not this? Many drugs of questionable value have been reported to be effective on inadequate trials.

DR. MARSDEN: Why not say, "Many drugs have been reported to be effective, but the trials are inadequate."

DR. BENENSON: I don't see that that is different, but this implies they may still be effective.

DR. SIMPSON: I think I have created a monster.

DR. BENENSON: Yes. I begin to think it is bigger than the problem.

DR. SIMPSON: I would suggest that the sense of this is agreed upon by all of us, perhaps, and maybe an

overnight stay will bring out the most felicitous phrase.

DR. BENENSON: Fortunately, we have an executive secretary who does not have anything to do for 24 hours. He only has the full meeting.

All right. Let us move on to the next problem. Colonel Cutting, does that essentially answer your question? Does it create more confusion to you?

COL. CUTTING: To be perfectly honest, sir, I think it is as close an answer as we are going to get. I think perhaps the most important thing that has come out of this is the fact that we have got to decide on a therapy on an individual case basis in terms of where they acquired the disease, therefore, which parasite they are probably infected with. We cannot come up with a single therapeutic regimen which can be applied across the board with uniform results.

DR. BENENSON: I think that is the sense of what I have been hearing. I think in some areas you would be grossly overtreating your patients unnecessarily.

COL. CUTTING: I think it is probably expecting too much as you suggest to have you run down every single area of the world that has leishmaniasis.

DR. BENENSON: Major Hendricks is all prepared to do that. He is revising the TB MED now that we have asked for it.

B. Is medical follow-up of all exposed personnel

indicated? Now, if we use Carl Johnson as a point of departure, he has a fairly short answer to this. All individuals who are stationed in endemic areas should be advised of the danger of infection with cutaneous leishmaniasis and of the methods of prophylaxis. They should be informed to see a physician should a skin lesion or lesions develop while in or after leaving the exposure area. The incubation period varies, but ordinarily the lesions develop within three weeks to two to three months after infection. However, any lesion developing within a year after exposure should be suspected as possible leishmaniasis, and serological follow-up is not recommended.

Now, Dr. Johnson brought me the Public Health Service yellow card that said essentially this. This is what I am talking about. You tell the traveler that if you develop any of these following symptoms, you call one of these telephone numbers or you go to your doctor and tell him to call CDC and tell them that you have got these signs and symptoms.

Okay. That is a whipping horse.

Dr. Schultz?

DR. SCHULTZ: The health alert card is rarely handed out.

DR. BENENSON: I know that. I know that, but it is the same principle. You tell the man, "Okay, you have been in

Panama. It is possible you are going to develop" -- or you are coming to Panama. While you are there, there are a lot of sandflies, and if you use a repellent there won't be any problem, and if you are out at dusk, you are in danger, and when you get out of here, if you develop a sore,a lesion that does not heal within a couple of days, go to sick call and tell them you have been in Panama in a tropical training exercise period.

DR. SCHULTZ: I did not mean to disagree with the principle. The principle is an excellent principle and should be done with other diseases like malaria.

DR. NEVA: I think the statement here about the usual incubation period, three weeks up to about two to three months, but then any lesion that develops within the year could be suspected of being leishmaniasis is a pretty good statement. I would agree with that.

DR. BENENSON: I think the whole thing is good. Dr. Simpson?

DR. SIMPSON: I think in view of the uncertainties and absence of agreement on prophylactic measures in general, especially the chemoprophylactic ones, it might be better to change that particular phrase, "methods of prophylaxis" to "individuals should be advised of the precautions that should be taken to avoid exposure to sandfly bites."

DR. BENENSON: All right. I think that is right. That is precisely what he was thinking of because there is no chemoprophylactic practice.

Dr. Takafuji?

MAJ.TAKAFUJI: Yes, two points. In essence this is what is being given out as information to troops who are going down to Panama now, certainly the ones from Ft. Bragg, but what I have question about is for example individuals who are going to other areas where we are talking about more serious forms of the disease, for example, Brazil, for example, individuals who are going to the Brazilian jungle warfare course where we know for a fact that we have seen much more disease, much higher attack rates. Is there an indication in these type of individual to do a little bit more in terms of pre-deployment evaluation and so forth as well as post-deployment evaluation?

CAPT. GUNNING: How much more?

DR. BENENSON: A lot more.

MAJ. TAKAFUJI: This is what I ask you.

CAPT. GUNNING: No, I mean how much more attack rates are you getting?

COL. NOWOSIWSKY: For the past 18 months five individuals went to Brazilian jungle warfare school. Of those five individuals, four have been located, and all four were discovered to have leishmanial lesions, cutaneous

leishmanial lesions.

DR. BENENSON: Developing frank lesions?

COL. NOWOSIWSKY: Frank lesions. They all had them when they were discovered.

So, the fifth one has not been located yet, but for all practical purposes you can consider 100 percent attack rate. They have been in the jungle anywhere from several weeks to three months.

DR. MARSDEN: This is Manaus, is it? The entymologist at Manaus at the National Institute of Research has four personnel, himself and three technicians, and the incidence in his staff is 120 percent. He has had two individual lesions, one on each elbow about three months apart. He has had it twice. So, the incidence is 120 percent.

DR. BENENSON: What therapy did they --

DR. MARSDEN: They took antimonials.

DR. BENENSON: But their lesions, and the lesions of your men were manifest lesions?

DR. MARSDEN: Yes. One wonders what the Brazilian military are going through there because you never hear anything in Brazil. How many of the Brazilian military are doing maneuvers in the jungle?

DR. WALTON: There was one published report, if you remember, of an airdropped battalion. What was that? That was 90-some percent.

DR. BENENSON: Isn't that asking for improved insect repellents and so on and so forth, improved indoctrination of personnel on how to avoid sandfly bites?

COL. NOWOSIWSKY: I would like to defer this question to Colonel Moussa who will give some information pertaining to our present plans.

COL. MOUSSA: I have been reviewing the information available in the literature about repellents and also some of the recent advances on this subject. I was able to come across -- just a little background here -- only one single test which was conducted with repellents in the Canal Zone which was done in 1958.

The report is not published. It is a manuscript, but because the results were inconclusive it was not published, but nevertheless, it was the only trial that was documented that I was able to put my hands on.

Now, we have heard, also, reports coming out from the field with some of our troops who are going through jungle training who are reluctant to use the repellent, indeed, which we have in the military system which is available for their use, and the reason which was offered that they do camouflage at night and they put this black stuff on their faces, and when they apply the repellent it smears and becomes soggy, and they just don't like the feel of it. So, to play warfare and games at night they would

rather be camouflaged than sticky.

So, we cannot tell for sure just to what extent the repellent has been used.

There are some recent advances which we became knowledgeable of. We do have cooperative arrangements with the Department of Agriculture at Gainesville which Medical R&D Command supports. This is the Army Medical R&D, and they have been experimenting with some new synthetic pyurethroids(?) These compounds are toxicants, by impregnating the fatigues, uniforms. They were testing those toxicants to see if they do indeed have some repellent action or activity. They did.

When they treated the repellents with this particular compound, the name of it is permethrin(?), in combination with the dye(?) being applied to the bare skin and they went in the salt marshes here with mosquitoes, right here in Florida, not only that mosquitoes were knocked down quickly, but after they moved into the area with their fatigues on, there was enough spatial repellency, this is in the atmosphere, that kept mosquitoes out of the area, not just mosquitoes, but they also got observations and records on biting flies, deerflies, horseflies and so forth which are very persistent biters. These are very gratifying because in their test they, also, tested to see how durable this treatment is in the fatigues. Those are the OD fatigues and they experimented with 100 percent cotton fabrics which

they were able to get. In the military we have 50 percent cotton and 50 percent polyester.

What is so unique about this is that they did some weathering testing by treating the fatigues and leaving them out in the open, and they tested these periodically to see whether they do remain effective, and they found out that after one month's treatment, weathering effect in the open, they remained 100 percent effective.

Also, they ran those in a special tumbling machine to see whether they can resist rinsing. They were able to demonstrate that the treatment was 100 percent effective against mosquitoes after 33 to 50 cold rinses.

They, also, took some patches of cloth, and they treated them with the same toxicant, and they put them out in a special cage, and they simulated rain, and they exposed the treated cloth to nearly the equivalent of 50 inches of rain. The material remained effective, 100 percent.

They tried that with soapy water. That is where the material did not pan out in the test. It withstood two successive soap and water treatments. I got in touch with the Department of Agriculture, our peers there, counterpart, and asked if they had contacted the company that provided them with the initial compound, this permethrin, and they believe that the soap wash treatment which was harsh on the impregnation test can be overcome, at

least that is their assessment, can be overcome with improved formulation, and the company now is experimenting with an emulsifiable concentrate formulation that probably could do the job. We are not sure.

So, another interesting feature, also, about this material, they noticed that if you use the repellent alone and walk in the salt marshes, you get a given protection time of maybe an hour or two hours. You get potentiation or enhancement of your protection time, a prolongation of that period if you wear the uniform with this treatment, and I believe it was an increase of nearly 50 percent of your total protection time on the skin on the exposed skin if you wear the treated uniforms.

So, we feel here that maybe this is something that ought to be looked into. We do not believe that anyone has tried to test these fabrics or these uniforms in the Canal Zone, and we feel maybe we ought to look into this.

All right, now talking about the toxicological evaluation of the material, while the Department of Agriculture is working on this, they were working on a series of compounds. Part of it was for development of an aerosol for this insecticidization of aircraft when we bring aircrafts from overseas.

A lot of the toxicology which is needed for all this series of synthetic pyrethroids has been turned over to

the Army Environmental Hygiene Agency. They do have a Department of Toxicology there, and they do provide under a memorandum of understanding between the Army and the Department of Agriculture, they provide them with this toxicological evaluation and support, and from all indications so far on the tests that have been conducted at the agency, the material does not irritate the skin. There is minimal absorption, and they do not envision any problem safetywise.

CAPT. BROWNLOW: The marines have had the repellent jacket for approximately two years now. It works very well, but Dr. Quinlan tells me they refuse to wear it because it smells, and they are afraid it will give away their position. It is impregnated with DIT(?)

LCDR. QUINLAN: The practical point that in the jungle warfare, that our marines are just concerned that the abnormal smell of the insecticide is a deterrent to their own health. They would much rather face the insects than believe that their own position is given away. It is just the practical point of the consumer.

DR. BENENSON: This, of course, is not new. Dr. Moussa?

COL. MOUSSA: I have had the chance when I was in Thailand when this problem of DET or the repellent being detectable by Vietcongs to look into this problem. I did some field testing right in the jungle of Thailand which is

comparable to what we had in Vietnam where we literally got natives and also US servicemen. All these people applied the repellent like we would recommend them to use it. We had them bunched up in groups and also in single file. We had sniffers for the test to see if, indeed, they can tell. We had to, of course, use controls with an ethanol or even plain water, and we could not demonstrate with any factual figures the ability of somebody to detect an odor. They couldn't detect it. The only way, when you have a whole company, maybe, in one given area and you are that close. In fact, there are some sniffers who failed to detect the repellent when it was about 10 inches away from their nose.

DR. BENENSON: The power of the rumor. In World War II, Atebrin was unused in many areas because Tokyo Rose said that it would produce impotence. It was not used. It sounds as though you have got something there that requires test, and I am sure that you are developing plans for it, one of which is that one of these tests of the marine point by giving it on these maneuver exercises with aggressor independent forces to see whether they can smell it. That is the answer.

Dr. Walton?

DR. WALTON: To get back to the question of follow-up, are you finished with that?

DR. BENENSON: That is what we are discussing right

now, the follow-up of the Brazilian.

DR. WALTON: Dr. Takafuji accounted their study. You definitely encountered lesions in troops who had not gone to sick call by conducting a physical inspection, and in the 1965 study we did after being alerted, having a certain number of cases in sick call, we had one of the old raincoat combat boot formation inspections in each of the battalions and cases were detected in all three battalions at that time.

So, I think that in cases where you have a complete unit with unusual exposure it might be appropriate after one month or so to conduct that type of inspection.

DR. BENENSON: How do you define that degree of exposure?

DR. WALTON: Known hyperendemic areas.

DR. BENENSON: Everybody in Panama?

DR. WALTON: No, I don't think that is true, but overnight bivouac, more than one day in a jungle training situation, and it is not all over Panama, but before we lost the Rio Atto(?) training area for example, there was much more training there than there was in the Canal Zone, and we were never able to confirm a single case from there for example. It is in certainly fairly circumscribed areas.

If you are not really familiar with the area you might not know where that is, of course, but certainly in the Canal Zone you know where the majority of the cases are

coming from.

DR. BENENSON: Maj. Hendricks?

MAJ. HENDRICKS: One of the problems, Dr. Walton, that Dr. Takafuji related to briefly is that in this calendar year alone we are talking about exposure of 10,000 people based on our current projected training sites. So, it becomes a logistical problem to round them up to look at them. It could be done.

Also, one of the other problems is emphasis from the top down to the medical personnel that examine these people, primarily the paramedical personnel. If a combat troop, the 101st or 82nd Airborne or some of the marine divisions turn in with something as minuscule as these small papules that we have seen they would be laughed at and sent back to the field immediately as a malingerer, and probably would have a very hard time getting any attention for something that small.

DR. BENENSON: I think that you are hitting on a slight feeling of mine, that we are overaccentuating the importance of the minimal lesion. Now, a major lesion is another matter. If he has a large ulceration, he is going to come in by himself, but we are looking for those evanescent lesions that are only found when you seek them.

Now, if you look at the statistics you will see where studies were done, and then the rates are very low

because those are the ones who come in on their own power because they have something they are worried about, and then this year we have a big peak again because 10 people were found, none of whom would have reported in at that time. I don't know what would have happened if they had not been treated.

The question has been made, how many of them would have cured themselves completely without therapy, so that if it becomes a matter of lining up 10,000 men during the course of the next year once or twice, this becomes a major task against which we always have the line opposition to taking troops out of their normal function. "The damn medics tie us up all the time," so that while I recognize that that would disclose many more cases, I question the practicality of it.

Dr. Marsden?

DR. MARSDEN: I think I would do a leishmaniasis skin test. I am surprised this has not been mentioned. I mean is it generally frowned on here or something? Because of all the skin tests, you know, it is probably the most reliable, and it is a pretty simple thing to do.

DR. BENENSON: Where do you get it?

DR. MARSDEN: We make our own in Brazilia.

DR. BENENSON: We do, too, but where does the Army get it.

DR. MARSDEN: The Army cannot use it?

DR. BENENSON: No.

DR. MARSDEN: Why not?

DR. BENENSON: Dr. Russell, do you want to comment on that?

COL. RUSSELL: There are two skin tests we have looked at in terms of availability. One is the soluble antigen preparation that was made by Parke Davis a few years ago for which we hold an IND and for which there is functionally no efficacy data and no way to evaluate potency. We are looking into the possibility of re-evaluating that, and the other issue had to do with the Montenegro type antigen, said to be made by Burroughs Wellcome, but several inquiries to Burroughs Wellcome regarding -- I think the first inquiry we asked them whether they would be interested in submitting an IND, and they said, "No."

We went back to them and said, would they take a contract to submit an IND, and we have not heard from them, and there seems to be a considerable reluctance on their part to respond, and looking into the rules and regulations under which biologicals can be administered to people in the United States, including soldiers, it seems to be very difficult to use anything that does not at least have IND status and to get a present-day IND on a skin test antigen looks to be a considerable undertaking.

DR. BENENSON: What data do you have to provide for an IND which is an investigational use?

COL. RUSSELL: You have to have some evidence of efficacy, some way of measuring potency and a reasonable estimate of its safety.

DR. BENENSON: Isn't that what you do under the IND, except for the safety? You would have to show safety before you could apply it to the men.

COL. RUSSELL: You usually have to have some measure of potency other than just human trials. One of the fundamental problems with all the skin test data is there aren't any large-scale controlled double blind trials to indicate efficacy in a situation like this, at least I have not been able to find any information that says that there is such a trial published. There are a lot of anecdotal ones and there has been a lot of use of skin test antigens in an individual clinical situation. I am not denying the usefulness to the clinician in making the diagnosis, along with other diagnostic tools, but I have been beating on my staff for the last two months to provide some data that supports the contention that a double blind study or any kind of a controlled study has been done with any leishmaniasis skin test antigen that evaluates its efficacy in terms of either diagnosing infection or diagnosing disease, and the literature so far has not produced such

information.

I leave it to the experts to tell us where we have missed.

DR. BENENSON: Brice, isn't there data relating Montenegro results with serological results? The correlation sometimes is bad. This is true.

COL. RUSSELL: Correlation with whose serologic results?

DR. BENENSON: Does that answer your question?

DR. MARSDEN: I understand why you have not got it. I think it is regrettable because I think this is one skin test in parasitic disease that has great value, particularly in leishmaniasis in South America. If that data does exist, hereafter, I would suggest two sources, Letisis and Roteberg from Rio, in which it was just about Montenegro antigen and then of course, have you looked in Leishmaniasis Tegmentarium Americana, Purcell and Beratta? It is a great big book. Oh, it is like this, where Purcell and Beratta go through all the studies. So, I think you will find that Purcell himself was one of the first men to -- he did a lot of schools. He was one of the first men to show, you know, that there were a lot of people in these schools who had never had any sign of leishmaniasis, but had a positive Leishmania, and you know this raised the whole business of could you get animal Leishmania producing positive results.

In our own practice it certainly is most valuable to us, in our area, and we use it all the time. I can imagine a very simple screening procedure like that would help you considerably, but if you cannot get it passed, what is the point in discussing it.

COL. RUSSELL: I don't think it is an impossibility. I suspect it is worth pursuing, but I don't think we can say now that it is a reasonable operational concept within a year or two.

DR. BENENSON: Dr. Schultz?

DR. SCHULTZ: I would like to nit-pick with Col. Russell on terminology. The term "efficacy" is usually applied to drugs, whereas sensitivity and specificity of the skin test would be more appropriate terminology.

As far as the availability of it, CDC has a number of skin tests that are made in the laboratory of Dr. Kaygin, skin tests for data in schistosomiasis and others.

We have never made a test for leishmaniasis. The only person that I have known of during the past 10 years in the United States who has had this test is Kevin Kaygin in New York City, and he keeps pretty tight control of it.

We have run into the same kinds of problems for producing IND's for the skin test, and it has impaired the distribution of these skin tests significantly because they were developed before the time of the stringent FDA and

Division of Biologicals' rules, and they were used, although not carefully studied for sensitivity and specificity, but in recent years we cannot send them out because no IND's have been approved.

DR. BENENSON: Dr. Simpson?

DR. SIMPSON: We tend in general, in our institution, to avoid skin tests because it will alter the serologic sensitivity subsequently. I wonder if that general axiom might not apply here with this particular group of people who are likely to be sent into endemic areas repeatedly over time, and it is hardly a one-shot deal. I wonder, Dr. Marsden, if this has been measured in your group, you know, the control population that has been skin tested?

DR. MARSDEN: I have got no definite information on that. The amount of antigen that is injected is very small, and of course, when we think about the development of this fluorescent antibody test, it takes some time even in patients who are infected several months, and in patients in whom we find organisms sometimes the test is negative. So, I would doubt whether that would be a problem. I would just like to add that there are two ways of standardizing the test. It is not just the nitrogen which is what we do. We do a nitrogen to microkelo(?) but Neale has recently published a method standardizing it in Henriettine(?) guinea

pigs by inoculation to the skin of guinea pigs, and if you don't get a positive response while to Ralph directly because I think Ralph would come across with some valuable information. This Kayhill material is from Wellcome.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: One of the things Colonel Russell was referring to specifically in the literature is many of these larger studies, earlier studies with WRAIR skin test antigens, Leishmania, have almost all been conducted in endemic areas. So, occasionally it might make some problems in interpreting the results because these people were not isolated or not moved from there to a non-endemic area or something. So, there are always those possibilities, and I know that one of the ones Kayhill mentioned of the possibility and actually later proved in the laboratory that lizard Leishmania, for example, would give a positive skin test.

DR. MARSDEN: Proven in the lab?

MAJ. HENDRICKS: By actually inoculating some volunteers with lizard Leishmania.

DR. BENENSON: Dr. Neva?

DR. NEVA: I don't want to prolong the discussion, but I think the point that Phil brought up is one that we cannot escape, and I would agree that the Leishmania skin test is clinically very useful, but --

DR. MARSDEN: The only one, old chap.

DR. NEVA: But how are you going to really do a study which will allow you to say that this is a specific test? It is one helluva job because what you would have to do is to go to a non-endemic area and inoculate in scores or hundreds of people with the skin test to show that they are negative. One side of the coin is to take proven cases and to show that they have a positive skin test. The other side of the coin is to take hundred of people who you know couldn't have been exposed to leishmaniasis, and how are you going to get the permission to shoot this into them and do it? That is where the rub comes in.

DR. BENENSON: Ft. Bragg has got the answer because you are going to send a battalion down. You skin test them before they go, and they ought to be negative, and then you skin test them when they come back.

COL. RUSSELL: What do you do with the information? What do you do with skin test positive people?

DR. BENENSON: Then you look for serological evidence of infection, too.

COL. RUSSELL: I understand the experiments. I am talking about an operational concept, not experiments.

DR. BENENSON: I think it can be done.

COL. RUSSELL: The investigations can be done, but Teris is asking operational questions and not

investigational questions, and that is the fundamental difference.

DR. BENENSON: If we cannot give him an operational answer tomorrow, we should guide him towards something which will give him an operational answer for next year.

COL. RUSSELL: Please differentiate it.

DR. BENENSON: That is right. We have to differentiate it. In other words, today, it is not available for use here. Now, to go back to Carl Johnson, if we all agree with B with a slight modification that we have made, he answers some of these things. He is saying that if you do a screening the diagnosis is based on them and updating the organism. He goes into details on how he abrades or aspirates and then he comes to the same point that Dr. Marsden has just been making and that is that Montenegro is the ideal technique, so that we come right full cycle back again, and we say, I think we are in the position of saying that the best technique that is available for screening for infection is one which is not available within the United States of America, and I think that the Board would recommend that whatever actions are necessary be taken to establish the validity of or to establish the availability for the Armed Forces of the leishmanial skin test.

Is that the sense of the Subcommittee after we have debated this?

MAJ. TAKAFUJI: The point to be made, sir, is that when we send 650 people down, that is a lot of people to screen. Of that 650, if you can identify, let us say 100 people that may have positive skin tests but certainly in that group you would have your cases, that is going to help us a lot at the MEDAC level to identify cases as opposed to screening 600 people and going through them dermatologically. So, the skin test does, I feel have value, understanding what Colonel Russell has said about the availability of the antigen and so forth.

DR. NEVA: I don't think it is going to help you in this particular instance. There is no evidence that you can get a positive skin test before you get the lesion.

MAJ. TAKAFUJI: I agree.

DR. WALTON: I just never got in any technical reports because the climate got a little hot about the use of skin test antigens, but we started in all good faith using the skin test as a diagnostic adjunct, and we had 73 patients in whom skin test antigen was used in a diagnostic battery, and we had, of the 65, of the 73, 65 were positive both skin test and serology.

We had eight in whom we had positive IFA's, skin test negative. We had one who was skin test positive, serologic negative. Incidentally, we never could demonstrate infection. I cannot explain it. We had one proven infection

who was skin test negative and serologically negative, and all in all when the series was complete we had about 90 percent skin test positive. Ten percent were skin test negative but serologically positive. However, we retested these people from one to three months later, and I think with one possible exception they had all become skin test positive.

So, a rule of thumb is that skin test positivity does not develop until sometime after the lesion develops.

DR. BENENSON: All right, but it is easier to do the skin test than it is to have every man strip and do a physical examination, isn't it?

Ernie?

MAJ. TAKAFUJI: I don't believe so.

DR. BENENSON: I am asking the people who are involved on the practical level.

COL. BURKE: In the operation of the skin test, remember, it is not an immediate test, and it is a recall of 651,000 people. It is very difficult to get those people back in a, well, we used a 48-hour period.

MAJ. TAKAFUJI: I think certainly at a post like Ft. Bragg you can do that.

DR. BENENSON: Dr. Schultz?

DR. SCHULTZ: It is arguably better to skin test than a physical examination. I would like to emphasize a serological test that if you have finite resources, I would opt

for investing them into improving the serologic test because in general for other parasitic infectious diseases the serologic tests are more precise than skin tests, and it has the beauty of taking something out of the patient rather than putting something into a patient which requires the IND and going through the whole --

DR. NEVA: It requires IND's to take something out of a patient, as far as permission.

DR. SCHULTZ: Okay, permission but not IND.

DR. MARSDEN: I tend to disagree on the grounds this might be the one exception to that general rule. There are various skin tests for parasitic disease, but the only one I am working with now is the Montenegro. It is a relatively simple test. There are risks to it. It is true. You have got to have a lesion, but the point is you could have a very small lesion that is healed, but even when you strip them you cannot find. So, if you have a physical examination and this aid, you would detect, I think cases that possibly would slip through the net normally if you just did a routine physical. I don't know whether Brice would like the FAT as well. Perhaps it would not hurt to have all three. I don't see why you cannot have the whole outfit.

DR. BENENSON: Major Hendricks? I will get to you, Brice, you are third.

MAJ. HENDRICKS: I have one question I would like to

ask if anyone has any particular answers to, which is time frame. We are talking about after exposure. In this particular group we have known time exposure of a two and one-half week period, for example. What time frame would you think that if they were going to become skin test positive they would become positive?

DR. MARSDEN: I think it is a bit variable because I think after all it is a manifestation of delayed hypersensitivity. I think there is a way in which people develop. The rate varies, but certainly I would have thought the great majority were positive after two months, but there will be some that perhaps might wait three or four.

DR. BENENSON: Do you agree?

DR. WALTON: Yes, I agree. I would say 90 percent. The other 10 percent --

DR. MARSDEN: And there are always some that you, unfortunately, nobody knows why, but they are not positive.

DR. BENENSON: Dr. Moussa?

COL. MOUSSA: I just want to add a comment here. Maybe some of you may not be aware of the fact that at the Letterman Army Institute of Research on the West Coast in San Francisco, a group there who are working on the Montenegro skin testing antigen, and they do have a protocol. The work is in progress. They do have an animal model which is sensitized, and they were able to elicit immediate

and delayed hypersensitivity skin response to the antigen. They have a method which they perfected there for producing the antigen en masse and purifying it which improved a great deal in that direction. They prepared several batches of the Montenegro test which they are now testing for shelf life, stability and going through all the things that Colonel Russell mentioned earlier about some of these problems that have to be satisfied for an IND.

So, this work is actually being pursued.

MAJ. HENDRICKS: There is another question on the time frame. Dr. Johnson mentioned that the majority of lesions would appear within as early as three weeks and a majority within two to three months. Is that a consensus of opinion that that is a fairly good time frame, for example, if it was simply a physical exam, that one-shot thing operationally to look at these people for evidence of cutaneous lesion?

DR. BENENSON: I did not quite get the critical point there.

MAJ. HENDRICKS: When would be the best time to look, if we were going to strip all these people, for example, to look for cutaneous lesions, if it was a one-time operation?

DR. BENENSON: Are you offering an answer?

CAPT. BROWNLOW: No, sir. The question is if we

only examine them one time, postoperational 600 army or 700 marines or something like that, and we can only mount up one examination period, when is the optimal time post-exposure to do that physical examination?

DR. MARSDEN: As late as possible.

CAPT. BROWNLOW: But we have problems, of course, like everbody else with the personnel moving on.

DR. BENENSON: Are you doing this for statistical purposes or for medical purposes?

CAPT. BROWNLOW: Medical purposes.

DR. BENENSON: For medical purposes, it would seem to me that, well, I thought we had answered it in the other question, and that is that a routine physical examination does not seem warranted, but for statistical purposes,yes, because you want to make sure you don't miss any lesion which might heal itself, but if it does not heal itself, they are going to come in on sick call, aren't they?

CAPT. BROWNLOW: You are recommending that we don't program a specific physical exam following deployment?

DR. BENENSON: That is essentially what I took this B to indicate that we had all agreed on, that we not do any routine physical examination of everybody who comes out of the Canal Zone or out of Brazil but let them know that if they develop a skin lesion to go in on sick call or get to

the dispensary and tell the doctor there that they have been in the tropical exercise in the Canal Zone or something like that, rather than take 10,000 troops and strip them and look at them. We have been talking about the alternative of a skin test, and at least I have looked at it as a practical procedure using the intradermal gadget on the jet injector which is not 100 percent. You will miss some, but at least you can process a lot of troops rapidly in that way and pick out those who develop sensitivity. If you want to go further, again, to me it seems to be largely statistical.

Dr. Walton?

DR. WALTON: We seem to have been talking about spontaneously healing lesions, and this is something that bothers me. I am not sure that I have ever seen one, and Carl Johnson feels the same way. I think this is rather a trap. It is a dangerous concept that you get lesions that do spontaneously heal, and many of these remain relatively small, and don't ulcerate for a long time, but if they do spontaneously heal, it is a matter of at least a year.

So, I can think of some specific examples where our policy of any skin lesion which had not healed in one month could be referred for screening for leismanial infection would work very well because we had two people with very insignificant lesions on the eyelid. We diagnosed these relatively rapidly by culture. By the time they were

administratively processed and treatment was started they had very nasty lesions, and to the point they very well could have lost eyelids and with threat to the eye if we had not made that diagnosis very, very rapidly. These were very insignificant lesions at the time they were picked up.

I think maybe I am also influenced by the era in which I was in the Army, you know, like World War II. Formations with combat boots and raincoats were fairly frequent. At that time in Europe scabies, lice and things like this were a problem, and the advantage of this type of examination is that you don't need professional personnel. You can train your unit medics to look for lesions. They can spot a skin lesion and sort them out. It is the sort of thing that can be done at reveille formation. I know the Army has changed, but I still think that this is more practical than lining people up and injecting antigens.

I have had some experience with skin test antigens. We did 10,000 at one time in the Far East, and even with a perfectly organized setup and doing them rapidly, it is laborious. It is a tough job, and I really think that we have had a couple of examples of how a quick physical exam can pick them up, and there are no legal complications at all in that procedure.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: That was one of the reasons I

persisted with the point because the physical exam we gave this one study battalion was four to six weeks after their return. We picked up 30 referrals, if you will, with lesions or papules, 10 of which were culture positive.

I don't know if at that time they would have been skin test positive. That was the reason I was asking.

DR. BENENSON: That is not necessarily inappropriate. I think that is a reversal of our discussion a little while ago. Is it the sense of the group that there should be a strip-down physical inspection at some time after troops come out of an endemic area?

Dr. Gunning?

CAPT. GUNNING: I think it is a question of your purpose. I think as you stated, if you are trying to find out how many cases there were, then I think you have to inspect them all from head to toe. If you are simply interested in what kind of disease morbidity you are producing, then you wait until they develop a lesion and come forward.

DR. BENENSON: I think from the military point of view that is the pertinent question. What are you looking for, the amount of infection or -- I mean, I buy the fact, Brice, that maybe they don't heal themselves, but with the exception of a lesion on the eye or on the eyelid, I don't know that it matters. What do you think, Frank?

DR. NEVA: Again, it depends on the kind of

leishmaniasis you are talking about. I think it is pretty well documented in the literature that you can get self-healing in leishmaniasis, especially in the Middle East.

DR. BENENSON: And in Costa Rica it heals itself.

DR. NEVA: I think for the other thing are we talking about research procedures or are we talking about practicalities of just people have been in the endemic area and then what? I agree. I think for research procedure you go through several kinds of screening techniques, but for just worrying about the possibility of leishmaniasis developing if you get a lesion and it fails to heal, then they come in.

DR. BENENSON: Dr. Marsden, you have your hand up. With the type of disease that you are dealing with, is there hazard in not recognizing it at its incipient stage?

Are we prejudicing the individual in any way by not aggressively trying to find these earliest lesions?

DR. MARSDEN: In my area, yes. I mean my responses are different to Brice's. I have a slide somewhere of three cases closely studied of self-healing, but let us take the area where we work, a very remote area in Bahia. There is 43 percent positive Montenegros in the general population, and that correlates very closely with scars. I mean the great majority of these have scars, and these people have access only to grasses. You ask them, and "I have been putting

grass on it, doctor, for four or five months, and then it healed." I mean of no therapeutic value at all. So, they are healing, and then if you take in the area the number of people with ulcerated noses of which there are quite a number, 30 or 40, I had a Cambridge medical student down there who went around to each house and asked them, you know, and we have got the data for the number of years or months after their lesion on their leg or arm healed their nose broke out, and it is amazing. I mean it stretches over a period of about 10, and I think there is one at 15 years, and then of course we have got some people with terrible noses, and they have just, you know, sort of lost their nose entirely, who have never had a skin lesion, to their knowledge.

So, in this area which is almost certainly not the same as the one that Brice is talking about. The answer would be, you know, we have to regard it very seriously which is why we treat everybody with three courses of antimony because we have got no way of differentiating.

DR. BENENSON: That is after they come to you with a skin lesion.

DR. MARSDEN: Yes.

DR. BENENSON: All right. Now, we are talking about those who don't have sufficient or a lesion that they recognize that we have to go look for.

DR. MARSDEN: As I say, I have cases where there

was no lesion, and obviously they are the extreme example. There are some with very tiny lesions. The people in the area I am talking about have no access to medical facilities, and therefore have never had any definitive treatment of their leishmaniasis.

It is interesting that the great majority of them have healed with a scar and never had any trouble. I just might cite six siblings who all got leishmaniasis at the same time. Five healed within the year with nice scars that have never broken down again, and one girl went terribly to the bad with tremendous, lost half a buttock, moved up into the nose, took away the nose, et cetera, all infected at the same time. I cannot believe it is a different species. She just responded in a very odd way.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: Several of these patients that we have seen already have been in therapy from this particular battalion. They do not have, and I don't think will have or develop obvious lesions. I think we caught some of these very early when it was only a small papule. It will be very hard to qualify a scar, a cutaneous scar on several of them.

DR. BENENSON: Dr. Nowosiwsky?

COL. NOWOSIWSKY: I just would like to make a brief comment. You know this training in the Canal Zone has been

going on for decades, at least since World War II, and I am not aware, and thousands of them, and I am not aware of any major health problems in those individuals who have come back, regardless whether cutaneous leishmaniasis has been diagnosed or not.

Now, I am talking only about that particular area.

DR. BENENSON: Dr. Schultz?

DR. SCHULTZ: One reason that you may not be aware of it is that when the troops are discharged into the civilian population, it is unlikely that civilian physicians will make this diagnosis and give correct treatment, and I don't think you could rely on the troops themselves to associate the skin lesion several months after going into the tropics with their being there even with the warning that they are given.

So, I would add that as one reason in favor of inspecting them after training.

DR. BENENSON: Dr. Walton?

DR. WALTON: This is one of these gut feeling type reactions, but if you noticed the bar graph on a number of cases diagnosed in the Canal Zone for a period of years, the first case in the US military occurred in 1932, and there was not another one for about five years, and there were one or two, very sporadic, and in 1955, it was the first time there was any number. And then it is not only in military.

Among the Canal Zone population, Canal employees, we see many more cases of cutaneous leishmaniasis in the last few years than was noticed before.

Now, whether this is due to more roads, more people getting out in the areas or whether something has changed ecologically it is a real good guess, but this rather vague impression is borne out by Panamanian physicians, Carl Johnson. Everyone agrees that there is much more cutaneous leishmaniasis now in the last 10 to 15 years than there was before. So, possibly, although we have not seen any secondary effects from the thousands of people we have put through, we might see it in 10 to 15 years. I mean, this is pure speculation.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: One of the recent scientific publications, National Geographic had an article about the Canal Zone, and it mentioned a factor which might come into this. They did not mention it in line with the disease entities, but they mentioned the cause of the tremendous slashburn(?) agriculture pressure up to the edge of the zone, completely denuding most of the natural forest habitat there that they have a lot of problems more recently with having to continually dredge the Canal. This in itself could drive more infected reservoirs into the area, not saying that they would continue to stay there because of ecological displacement

and so forth, but it could build up an increased incidence in the reservoir host as another possibility.

CAPT. GUNNING: I was just going to say I think that is the natural history of Leishmania infection, that if you want to study a pocket of Leishmania you go to a place where there is a resettlement area.

Certainly in Costa Rica the transmigration areas were the hottest bed, where people were cutting down jungle, where they accumulated a lot of brush on the edge of the clearing. The rodents begin to infest the brush pile. The sandflies are there, and then people stumble into it, and I am sure the same thing is happening in Panama because the population is exploding.

DR. BENENSON: I think we are going to have to move on this. I see enough diversity that I will ask for a specific opinion. Do we recommend a what do you call it, raincoat and boot inspection at some time after troops come out of an endemic area?

Dr. Farah, yes or no?

DR. FARAH: I think in the final analysis, yes.

DR. BENENSON: Dr. Gunning?

CAPT. GUNNING: I will vote for the marine contingent that is going down there. I would be willing to examine them all.

DR. BENENSON: You are saying, "Yes."

CAPT. GUNNING: Yes.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: Yes.

DR. BENENSON: Dr. Neva?

DR. NEVA: No.

DR. BENENSON: At least we don't have unanimity. Dr. Schultz?

DR. SCHULTZ: Yes.

DR. BENENSON: Dr. Simpson?

DR. SIMPSON: I would vote strongly affirmatively, but at the same time suggest that we recommend a thorough review of the skin test problem. You might want to approach that later.

DR. BENENSON: Brice, I think I know your answer is yes.

I think the yeas carry it.

All right. The next question that Maj. Hendricks specifically asked is when would be do this.

Dr. Farah, I will start with you. When would you do it? This is after they come out of the area.

DR. FARAH: I think it is a very, very difficult question to answer because it depends on the size of the -- sometimes you will get your lesion in three weeks. Sometimes you will not get it for a year. So, I suggest that this would be done at the latest possible time before they

leave the Army.

DR. BENENSON: Leaving the control?

DR. FARAH: Yes.

DR. BENENSON: In other words, up to a year or five years?

DR. FARAH: I don't know about five years. I don't if the incubation period is that long, but certainly within eight months to one year.

DR. BENENSON: All right. Wouldn't you want to pick a more specific time? In other words, if he is developing a lesion, the implication I get is that yes if he has a lesion, we would want to give him therapy. That is why the majority of you said, "Yes, you do want a physical examination."

Now, if the majority of lesions will be manifest in three months, wouldn't you want at that time or maybe make it four months, at that time, to do a physical examination to detect the lesion, rather than wait until eight months or something?

DR.FARAH: Yes. I would take a specific period, if I had to put one date, as maybe three or four months, at which time I would --

DR. BENENSON: Three to four months.

DR. FARAH: Or maybe even three to six months because that would be the peak at which you will get these

things. Again, if I carry from the experience in the Middle East, for instance, the peak of the sandfly population and the peak of incidence of disease vary between the period of three and six months within a year.

So, if we are really looking for the majority of cases, I would put that about that time.

DR. BENENSON: In other words, one might say that what we recommended before that all troops be advised if they develop a skin lesion to report to sick call and then at six months you check out those who have not come in with a lesion.

Major Hendricks?

MAJ. HENDRICKS: I should know, but I don't know about the Army training cycle. I do know about the Marine Corps training cycle. The jungle warfare training and commitment to the Caribbean area culminates or plateaus their training as an intact battalion. Once they return to the United States they would lose 50 percent of the people immediately and start replacing them. I don't know if that is true with the Army, the 82nd, 101st and so on. So, you would lose half of the population almost immediately from a control in that particular battalion.

DR. BENENSON: There is nothing you can do other than warn them if they develop a skin lesion.

Dr. Gunning was going to do it, but he cannot --

Dr. Brownlow?

CAPT. BROWNLOW: You could make a health record entry when everybody is together and also alert your individuals that six months hence --

DR. BENENSON: All right. So, then we would leave it that they would be examined at six months, period, I mean how the services work it out; is that feasible from your point of view?

COL. NOWOSIWSKY: It is, except I would give us a little bit more elbow room, such as when this is not feasible that we instruct those individuals to be on the lookout for the lesions, so that not to make it so that in each instance we have to have this inspection, because it may not be possible in each instance.

DR. BENENSON: I am a little unclear on how one would phrase that. Are you saying that you would rather have it in a period of three to six months, rather than say, "At six months"?

COL. NOWOSIWSKY: Oh, yes, a range rather than --

DR. BENENSON: Right.

COL. NOWOSIWSKY: But then also leaving some room, saying that in those instances where such inspections are not feasible just to --

DR. BENENSON: The Navy says that they will put it in the medical records so that they will be looked at at six

months. Can you not do that?

COL. NOWOSIWSKY: It is one thing putting it in our medical records --

DR. BENENSON: I know, but what more can you do?

Major Hendricks?

MAJ. HENDRICKS: Sir, I think it probably would require even to get it into the record, a formal request from higher authority before even the records were tagged, if it was to be done.

DR. BENENSON: Brice?

DR. WALTON: I am relying on my memory without going back and checking the records, but all of these cases, for years we recorded the date of the most probable exposure, and I would venture to say that by 60 days after exposure you would have 80 to 90 percent of the lesions recognizable already. Do you remember that, Jim? Wasn't that it, at least 80 or 90 percent by 60 days, and most of them have something recognizable? Probably the mode for reporting to sick call is about six weeks after exposure.

DR. BENENSON: Could it be the lag period between incipiency of the lesion and recognition that it is chronic and they need therapy? You have got a six month lead which is three months slower.

DR. FARAH: Maybe the biologic behavior is a little bit different between the two, obviously, and even

in experimental situations, in animals using L. tropica in mice and also in things which were done in the early 1940's you could relate between the dose or inoculation and the incubation period. Let us say in the mouse if you give 100,000 organisms you may get six to nine weeks before a lesion develops, whereas if you give 2 million organisms the lesion will appear within three weeks, and the same similarly in the findings in the human, but the interesting thing about L. tropica is that when there is a peak of Phlebotomus, the peak for fresh lesions in the area follows by about three to six months, and this is very true for the new settlements, you know not the urban type but where there has been exposure out in the field rather than in the city.

DR. BENENSON: All right. Let me try this for size. In addition to what we have here, we will add, "When feasible," and this satisfies your desire, "physical inspection for skin lesions should be carried out three to six months after the individual leaves the endemic area."

DR. FARAH: Do you want to put the thing about instructing?

DR. BENENSON: Yes. In other words, this paragraph we have here would stand, but then we would conclude it when feasible physical inspection be carried out.

COL. NOWOSIWSKY: Dr. Benenson, even if you would add to this that health education of personnel prior to --

DR. BENENSON: This is what we tried to say here. They should be advised of the danger with cutaneous leishmaniasis and --

COL. NOWOSIWSKY: Oh, that is with the previous paragraph?

DR. BENENSON: Yes, and be instructed in methods to avoid -- you know, that was all included. This is just adding this sentence at the end of what we had before. So, we are now recommending when feasible a physical inspection be carried out.

Yes, Dr. Simpson?

DR. SIMPSON: I certainly agree with all of this, and I would particularly like to concur in the use of Colonel Walton's hard data from Panama that would indicate probably during the third or fourth month would be the ideal time, but regardless of the arbitrary time that we set on this, it seems to me that strong consideration should be given to a rather formal attempt at troop education without creating a whole lot of anxiety. At least an effort should be made to inform the troops about the problem, what this will look like and --

DR. BENENSON: I think we have already recommended that they be indoctrinated in what it is and so on and so forth. I think that is covered, and how to avoid exposure, minimize exposure. Maybe Dr. Moussa's uniform will come along,

and then we won't have to worry about that.

Dr. Schultz?

DR. SCHULTZ: About this indoctrination business, I would say that it probably would not always stick with a person because of limited understanding of the time span that elapses, and I would say the cure for that would be the concept of the health alert card, either giving it to the soldier himself where he could put it into his effects and hold on to it for future reference or tagging it on to his health record, saying that he has been exposed to leishmaniasis, so that if somebody examines him six months later the physician will think about it.

DR. BENENSON: I think that is what in essence Captain Brownlow was suggesting before. I don't know that you need a recommendation from the Board to that effect though, do you, either service? The Air Force, I guess, is not concerned with this problem. They don't do jungle training.

DR. SIMPSON: The term "indoctrination" has a very specific connotation, does it not, in the military? It really means a program, not just word of mouth?

DR. BENENSON: No, what is implied, we did not use the word "indoctrination" at least if we use what Carl Johnson has written. It says that all individuals should be advised. Now, the services in their health education

program, I think ought to know how to proceed on that. I am not sure that it is our function or I don't feel that it is our function to tell them how to administer the advice to the troops. I think the important thing is that they so do.

Do you need anything?

COL. NOWOSIWSKY: No, this thing, once we have the Board's recommendations in the broadest sense, then we go as far as implementation and try to tailor make it to specific requirements to specific units.

DR. BENENSON: All right. Now, what I had written for appropriate screening techniques, we may want to change now, but let me read what I had written because I have labored through writing it.

For follow-up studies, the most valuable procedure available today is by the use of the Montenegro skin test. However, this is not available in the United States at the present. It is recommended that appropriate steps be taken to establish the availability of the test for military use.

It would be of value in two to four months after exposure. I am not sure that is true. I think that is too short.

COL. RUSSELL: I just wonder what the data base is for the value of the Montenegro skin test antigen in the screening as a screening tool. Is there really a data base?

DR. BENENSON: I don't think so. I don't know.

DR. MARSDEN: You mean a situation like this?

COL. RUSSELL: Yes. Is there a data base for the value of the skin test antigen in a large number of non-immunes that have been in and out of an endemic area, and of what real value is it in terms of predicting disease and infection or estimating disease and infection rates?

DR. MARSDEN: Of course, what it is is a delayed hypersensitivity test to a specific antigen. It is a sensitive one as such. It will give you some indication as to whether the patient's body has horbored that antigen. That is what it tells you.

COL. RUSSELL: In theory. I just have not seen the data that tells both the specificity and the reliability.

DR. MARSDEN: There is a lot of data on specificity. We have tried an awful lot of things. Some of the most recent studies are Brice's in Ethiopia. He is very fond of it, but you see you are quite right. The trouble is it has always been used in an endemic area, as Frank says.

DR. BENENSON: On a clinical case.

DR. MARSDEN: In a population to try and determine what exposure --

COL. RUSSELL: None of them have been double blind.

DR. MARSDEN: In these areas there are so many things to do.

COL. RUSSELL: I understand that. I am just trying

to evaluate the basis of your recommendation. That is all.

DR. MARSDEN: You are perfectly right.

DR. BENENSON: I read this, and I prefaced it by saying that I am not sure that it is any longer valid, and I don't think it is valid because we have recommended a physical inspection. If we have recommended a physical inspection, there is no need that I see to add to that a skin test for the clinical use. Now, again, from the research point of view to try to find out who might have -- Brice does not think any of them would heal themselves, but if we want to find out who might have healed himself, that might give us the answer, but that is research.

So, unless somebody on the Committee objects, I am going to strike what I just read and say for C here that the most appropriate screening technique would be the physical inspection.

MAJ. TAKAFUJI: You, also, talk about screening of people with skin lesions, because okay, we have picked up now people with skin lesions. Now, how many of these have leishmaniasis. The skin test is still --

DR. BENENSON: That is not screening any longer, is it? That is diagnosis.

MAJ. TAKAFUJI: It is screening of the lesions that you pick up.

DR. BENENSON: If that is the interpretation, then

the screening should be the demonstration of the parasites, and actually Carl has gone into that in detail. In other words, if you have a skin lesion, then what you want to do is demonstrate the parasite, either by smear or by culture or biopsy or whatever technique you want. Then you establish your diagnosis. The others are peripheral because the Montenegro at this point might represent infection 20 years ago when they were a kid in that area.

Major Hendricks?

MAJ. HENDRICKS: Colonel Walton mentioned at least one case that I remember in pointing out there a minute ago. How many subclinical cases might you have that might not be detected on a basis of just physical exam? He pointed out, I think, at least one case or maybe it was eight, I don't remember, where there were skin test positive people, and Dr. Marsden has related a skin test positive with no evidence of cutaneous lesion. You would miss those on simply a physical exam alone, but they could have subclinical cases.

DR. BENENSON: All right. Let me ask what you do if you find them? Are you going to treat them? It is going to be another statistic.

DR. MARSDEN: It might be worthwhile following those people a little more closely. It is not known as yet in various parts of the world this phenomenon has been

recorded, skin test positive and no evidence of lesion, interpreted in East Africa as lizard Leishmania which was mentioned, I think. I mean we don't know whether this is -- I, personally, have never seen anyone who has had a skin test -- I have not been there long enough, but I have not seen anybody who has had a skin test positive and then suddenly broken down with galloping leishmaniasis.

MAJ. HENDRICKS: But you did mention, I think that you had seen skin test positive that had no obvious scars.

DR. MARSDEN: Yes, oh, a high proportion.

DR. BENENSON: And the thing I am wondering is 43 percent of your population is skin test positive. What are you going to do about it? Are you going to treat them?

DR. MARSDEN: A great majority of these have got scars. They have had leishmaniasis. You ask them and they say, "Yes, I had this on the leg." Some of them have not. I cannot do anything about them. They are in the interior. They are better. It is the ones in the nose that worry me.

DR.BENENSON: Dr. Simpson?

DR. SIMPSON: Would it be within the province of the Subcommittee to recommend exploring the feasibility of a skin test trial, a highly controlled skin test trial on a previously unexposed, non-immune people who undergo a brief exposure of two or three weeks duration just to see what seroconversion does occur?

DR. BENENSON: That was what was encompassed in the sentence I had written that said, "It is recommended that appropriate steps be taken to establish the availability of test for military use." That is what you would have to do. You would have to skin test people who have not been exposed to establish the specificity. In other words, they should be negative at that point, and then I don't know how you would really establish the sensitivity. You skin test them when they come out, and then if you find these like Brice, do they have disease or not or do they have infection. If the other serologic tests become positive, then I think we would presume that they were infected.

Now, the thing we are not discussing or have only alluded to, and I have not written in here is the development of more reliable serological procedures. We have a serological procedure which we think is reliable in Panama but not reliable in Washington. That means that we need more reliable serological procedures.

COL. DIGGS: Is it reliable in Panama in two months?

DR. BENENSON: Skin test or serology?

COL. DIGGS: Serology.

DR. WALTON: I don't know what the total size of our military population statistics is, and this was not a single test. Some of these people came back to the clinic

three times, but to my knowledge I can only think of one person that remained serologically positive in parasitologically proved diagnosis.

DR. BENENSON: Positive or negative?

DR. WALTON: I am sorry, remained negative. So, in essentially 99 point something percent of the people who have had lesions that were proven, they were serologically positive.

COL. RUSSELL: IF we repeat it enough, we will get all of them with Carter's test.

DR. BENENSON: I suggest you get Brice to run some of your serum for you and see whether the Panamanian test is different from the Washington test.

COL. DIGGS: We already did.

DR. BENENSON: Major Hendricks?

MAJ. HENDRICKS: I think, Carter, what you asked them was at two months' exposure or two months known infection.

COL. DIGGS: Yes, two months after exposure.

DR. BENENSON: You still have the problem that 20 percent of yours were positive before they were ever exposed. That is the weakness in your serological data. There is no specificity. I don't know what a positive means.

DR. WALTON: We get about 2 percent in our normal population overall.

DR. BENENSON: Dr. Gunning?

CAPT. GUNNING: I was going to say that I think there is a terrible problem with what I perceive to be as follows: A lot of people are under the impression the same as Dr. Walton, that he has never seen one heal spontaneously, and I want to go on record as saying that I have seen one heal spontaneously.

Now, that one was right here in the United States, and I know it, in front of my eyes. How many others healed spontaneously that I never knew about in an endemic zone? It is a totally unknown figure, neither known to me or people working in that area. So, when you take a group of people, young people, such as we are going to be putting into Panama, I think you are developing two problems. I don't think you have any idea how many have lesions that are so non-descript and non-specific that you will never know they had the disease which I think is kind of important from an epidemiologic point of view, and the second problem is that many of our young people have such multiple skin lesions. For instance, they get into the tropics and get acne tropica, and you have a chap coming down, and you think you have a lesion. Which one do you culture, and the money basically is in not culturing at all unless you have got real evidence of ulcerative breakdown or skin test positivity, and then you can really go after it. Otherwise you are chasing your

tail, and I see a great need for developing the skin test, and I don't think it is going to happen this year, but I really would like to make a statement in here that I think it is an important test. I don't think and agree with Dr. Russell that there is no data that uses it as a sort of a prospective affair. It has always been used in my experience as with Dr. Marsden to verify the fact that this lesion is indeed Leishmania, but yet it is a highly specific test because we have enough corroboration with culture positivity and the good delayed hypersensitivity that it does produce to say that it is a good test, and I think it would probably be a good epidemiologic tool, but that has never been really tested that way to my knowledge. I think there is only one way to do it, and that is do it.

DR. BENENSON: Dr. Marsden?

DR. MARSDEN: Actually we do have data which I will send if you are interested from this study area because we have now done 1,300 of Montenegros there, and we have the correlation with skin scars and history of leishmaniasis, and it is a very strong correlation. The discussion stimulates me to do this in an area where there is no leishmaniasis which I have as well, and I could do.

I am glad this point was raised because I do feel it is important to put on record something about the skin test. Your situation is benign at the moment. I cannot

regard this, if you will excuse my saying so as very important at the moment, but it could become very serious, and the value of the skin test, it could give you some idea of degree of exposure of a group of men. I think it would be unfortunate if it was lost sight of. We cannot implement it at the present time.

DR. BENENSON: All right. Let me try the modified draft here. For follow-up studies the most promising procedures available today are immunological, the Montenegro skin test and serological test. However, these are not -- the skin test is not available for use in the USA at present.

It is recommended that appropriate steps be taken to establish the availability of these tests for military use.

We have got to straighten out the IFAT discrepancy here and the other thing is that certainly anybody who works in those areas, everybody that I know who has been handling leishmaniasis is sold on the Montenegro test. That means that work has to be done to validate it, if in the sanctum sanctorum, the USA, the FDA says, "It ain't no good." So, we ought to test it out. If it ain't no good, we ought to tell the rest of the world. I think you have, in the military here, with troops going into the endemic area the opportunity unlike any other to evaluate it. If you cannot get it anywhere

else, Brice will make it for you in Panama.

All right. Does that answer C? No subsidiary questions are posed.

All right, D. Question, Dr. Marsden?

DR. MARSDEN: It is not a question. It is just a point. As I mentioned, we use the hamster a great deal. The hamster is not mentioned in this part here. I am sure you know, that obviously the culture is working extremely well in the material you have here, but certainly from the Brazilian platéau you find yourself forced onto the hamster I think as a procedure for trying to isolate the organism if you are really keen on getting it, so that where there is this business as we are following Johnson' thing --

DR. BENENSON: We can just add in here --

DR. MARSDEN: Hamster inoculation will be appropriate depending on the area at the end.

COL. BURKE: Rather than giving the hamster especially, wouldn't you want to say, "Animal model," rather than the specific hamster.

DR. BENENSON: Yes, it could be a mouse. Injection of appropriate laboratory animals.

All right. Then we go on to what is the risk of transmission of infection in blood donor programs and what preventive measures are appropriate. I have two opinions on that. I have Carl Johnson's, and I also contacted

Pena Chavarria in Costa Rica who of course has been dealing primarily with mexicana but has had -- what is he, eighty-odd years old now, and he has a tremendous experience with leishmaniasis. So, I think if we can get this one distributed, the essence of the answer of both of them is they ain't never heard of it or thought of it. Pena says that bloodstream infections of note -- I am having trouble with his English. A person with manifestation of leishmaniasis would not be acceptable as a blood donor. In other words, if he has a manifest lesion. He is assuming he would not. As far as visceral leishmaniasis, he says he ought to have a fever if he has a parasitemia and Carl Johnson has never been concerned with it or thought of it. Now, actually the practice of the blood bank in Panama may be pertinent, and that is that every donor unit that is used in the Santa Thomas Hospital a sample comes actually to Dr. Johnson who screens it for Chagas' disease but has never looked at it from the point of view of leishmaniasis.

Until this query came in, the thought had never arisen, and I am curious to know why the question was raised.

COL. NOWOSIWSKY: There were several thoughts, first of all some animal studies that were performed, I understand that the organism was obtained from the heart through intercardiac sample. There were also that the skin lesions would appear if they spread along the lymphatic

chains, and then there is a real possibility that it will get into the bloodstream, and what should we do if this thing happens?

We were mostly waiting to see what the group of experts to express their opinions, since you may have a real possibility of a number of subclinical cases with no manifest lesions, and what do you do in case you do have some risk.

Now, this would be an economic problem for us because taking, for example, Ft. Bragg, we collect monthly anywhere between 250 and 500 units of blood. The combat units are the primary donors of blood, and therefore we would be faced with a large number of units who are drawn from not being contributive.

DR. BENENSON: There are no instances that were brought to your attention of someone who developed the disease without having been in the endemic area, in other words, and had received a transfusion. There is no case that instigated the question? None in your records?

DR. SCHULTZ: None that I know of, but Marty Wolfe who was here this morning, I believe is the author of a paper on blood-borne protozoan diseases other than malaria in a review that was written about four or five years ago. We could check with him.

DR. BENENSON: Dr. Marsden, what is your experience

with it?

DR. MARSDEN: Let me say that I would agree entirely with Johnson. I have never heard of a case in the literature. We have never seen one in Brazil. Of course, it is well documented with kala-azar in India, not transmission by blood transfusion but the presence in the peripheral blood of parasites. It was the way Colonel Short used to choose his laboratory technicians was to in fact present them with slides, and the first one who found circulating Leishmania in a case of kala-azar got the job, but I agree with what Johnson says here. I think he has done very well.

DR. BENENSON: Dr. Farah?

DR. FARAH: With cutaneous leishmaniasis due to tropica this has really not been a problem. The parasite is purely a tissue parasite, and there is no evidence of its transmission by hematological route, except possibly in the anergic type of disseminating cutaneous leishmaniasis where lymphatic or leptogenis(?) spread could be possible, but basically it has really not been a problem. We have never looked for it, but I am not aware that anybody has demonstrated it.

In experimental animals which follow very similar picture as in the human Leishmania tropica could spread by hematological spread in certain species, let us say the mouse in which you can find by injection of the skin parasites

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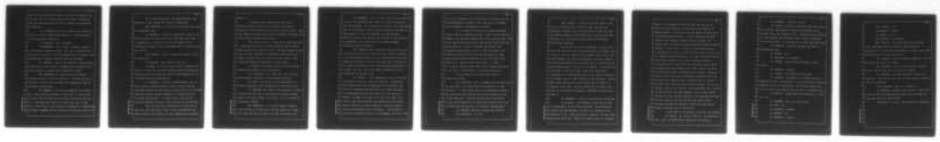

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that are in various other organs, including the spleen, and you don't see it in other species that behave differently like the CVA mouse where the lesion remains only localized in the skin.

So, I suppose in the final analysis the answer is that it is not a problem unless there is a specific defect in the particular individual.

DR. BENENSON: Major Hendricks?

MAJ.HENDRICKS: Sir, I have a question, playing as the devil's advocate on this. How can you document that fact that it had been a blood transmission in an endemic area? Consequently, possibly none have ever been reported.

DR. BENENSON: This is the question I asked before. Has there ever been a case of leishmaniasis acquired in the United States in someone post-transfusion, period?

MAJ. HENDRICKS: Dr. Schultz kind of alluded to something similar to this a while ago. Once they got out of the Army I am not sure they would be recognized if they had cutaneous leishmaniasis.

DR. BENENSON: You know my background is military, and I think military medicine is damn good. I, also, have to admit there are some good clinicians outside of the Army, too. They might not think of it right away, but if they have a lesion that is as refractory to therapy as leishmaniasis, ultimately somebody who has served in the Army will think of it.

So, it would seem that, now, take Brazilia, take Costa Rica, take Panama, they would be recognized.

MAJ. HENDRICKS: But how would you know it was from a transfusion, sir?

DR. BENENSON: If this is someone who lives in the City of Panama and never been out of the city, and he had a transfusion, he would have had to have gotten it by transfusion because he was not anywhere where a sandfly could get at him.

CAPT. GUNNING: Most of their blood source is from the same city.

DR. BENENSON: Yes, that is true, too.

DR. NEVA: If you are going to use a strawman like this, you could make the same hypothesis about schizophrenia, that how can you be sure some cases of schizophrenia are not transmitted by blood transfusion.

DR. BENENSON: I am not a leishmaniasist, but at least reading something about it, what I read is that the cutaneous strains do very poorly in culture media at 37 degrees, that the visceral strains flourish at 37 degrees and the cutaneous strains don't. In other words, they are heat sensitive strains, and that is why they grow on the surface.

Now, the thing that disturbed me a little bit is that yet, the literature is that it was isolated from the heart blood of wild animals and their temperature is usually

above 37.

I remember blood studies that were done on perfectly normal human beings who got in the hands of dentists, and the dentists did not even pull the teeth. They just rocked the teeth and did blood cultures at the same time, and the blood cultures were positive.

Now, these animals, at least what I read from some of these people working in British Honduras and in Brazil, these animals have lesions on them. They have nodules on the tail, and they undoubtedly are palpating them from top to bottom, and then they put a needle in their heart. They could have created a parasitemia at that moment.

DR. MARSDEN: But these animals you refer to were experimental animals in your laboratory?

COL. NOWOSIWSKY: No, these are, sir, from your distinguished laboratory of Christiansen and Herrar.

DR. NEVA: Oh, this is the old observations of Marshall Herdicks on the isolation from the spine in rat.

DR. BENENSON: Yes. There is one species, I forget it, in Brazil that the skin is positive and no known lesions, wherever you take a biopsy of the skin, but it is primarily a cutaneous disease.

MAJ. HENDRICKS: Dr. Zeledon recently reported in the Journal of Parasitology from the pocket rat, blood taken from the heart in Costa Rica from wild pocket rats.

DR. BENENSON: Yes, I am not saying that you cannot. I say if you manipulate the animal you create a bacteremia and a parasitemia. You manipulate man and you get a bacteremia. Now, it is transient, and if you put a needle in the vein at that moment you will get organisms, but here we have a situation where no one who has been working in the field has any knowledge of any implication that there has been transfusion induced disease.

Now, somebody with a great big lesion and his nose is falling off and so on, obviously never would be accepted as a blood donor, and what you are concerned about are these cases with minuscular lesions that we can only find when we do a physical inspection, and I think Frank's hypothesis is a pretty good one. How do you know schizophrenia is not transmitted or anything else?

DR. SIMPSON: I was responsible for the blood bank at Cocosolo for about a year, and actually I talked with Carl Johnson about ways of detecting all the things that could be transmitted through blood, and indeed the matter of leishmaniasis did not even occur to us at that point. However, it seems to me that all of the logic that I can bring to bear would indicate that especially with minimal lesions that are not easily detected, minuscule lesions that any parasitemia would be ephemeral. The biomass of circulating parasites would be infinitesimal, and that in the

case of an accidental blood transfusion with these that the reticuloendothelial system of the host would be depended upon to eradicate any wandering parasites.

So, it seems to me the risk is indeed extremely minimal with the cutaneous strains. I don't really know the answer to the donovani problem, but having said all that, there is one very curious observation that we have seen today that would point to at least a transient parasitemia with Leishmania braziliensis and that is the odd finding that lesions frequently localized around the knees at places where people scuffed themselves and the one report where there was a puncture wound with a briar. I think Dr. Takafuji showed us that which might indicate that there was at least a localization of blood-borne parasites.

Had you heard of this particular kind of observation before?

MAJ. HENDRICKS: Sir, I brought that up specifically for that point to be possible to be brought out in this discussion. In the 10-day course of convalescence between the second and third therapy the original lesion site was on the right calf. When he reported back at the end of 10 days convalescence he had this briar scratch on his left leg which was then positive for cutaneous leishmaniasis.

DR. BENENSON: There was nothing there before?

MAJ. HENDRICKS: No, sir.

CAPT. GUNNING: I will add one more case to that, one of our Taiwan cases, the first case, the lady who developed a lesion in her left antecubital fossa later had the typical lymphatic enlargements and a lesion on her left shoulder. It was treated at that point with Pentostam. The lesions regressed.

She went back to her village where I told you we found two cases now, and no other sandflies, and within about a month's time she returned with another lesion now on the right shoulder, but even more important than that, she had a third lesion which was on her left hip in an area that she would never expose to the bite of a sandfly. I was perfectly convinced at that point that I had seen a blood-borne transmission or recrudescence much in the same way that they are talking about in this fellow with the scratches. Whether the trauma produces the locus minoris versus stenti(?) or whatever it is, but I really do think that it occurs, but I think it is extremely infrequent. Otherwise we would have seen it.

DR. BENENSON: I think it is perfectly conceivable.

If we think -- the question is what is the risk of transmission in blood donor programs? What preventive measures are appropriate? We know of no instance of any suspicion of it. There has been no instance. You say, "How would you prove it?" There is no need to prove it, because

there is no instance of suspicion that has come up, so that the other thing is that yes there are evidences of generalization. We see rare cases in Panama in which there is obviously generalization. Usually they have had some damage to the lymphatic system curiously enough. So, it occurs. This is something perhaps associated with the febrile episode. I don't know. Is there a fever when they have a parasitemia, but if it occurs how often does it occur? We are dealing with probabilities. You take a perfectly normal blood donor, and he can be in the incubation period of Q fever because I can document the perfectly normal health of people with rickettsemia with Q fever lasting for about six days before their temperature first goes up. There is a report in the literature of isolating influenza virus from the blood in perfectly normal individual who in the next 24 hours later came down with clinical disease. You can go through a host of all possible viral infections that a person can have and not show any symptoms. Here we have another instance of that, and does anybody on the Subcommittee think that the risk is such that the military should apply a screening procedure to potential donors who have been in Panama or whether just to ignore it?

I will start at the bottom of the list, Dr. Walton?

DR. WALTON: No, I don't think so. As a matter of fact, there is experimental evidence to the contrary.

DR. BENENSON: Contrary to what?

DR. WALTON: Contrary to the fact that there should be a hazard, experimental evidence with hamsters using visceral forms and cutaneous forms. Cutaneous forms injected in large numbers into the heart blood caused no infection. It did not survive. It filtered out in liver capillary beds.

DR. BENENSON: You would say that the risk is hypothetical only?

DR. WALTON: Yes.

DR. BENENSON: Dr. Simpson?

DR. SIMPSON: None, except exclusion of overt lesions.

DR. BENENSON: Schultz?

DR. SCHULTZ: The question is the risk?

DR. BENENSON: The question is whether there is a significant risk of transmission to the point where preventive measures should be established by the military before they use as a blood donor someone who has been in an endemic area.

DR. SCHULTZ: Judging from the discussion, I would say, "No."

DR. BENENSON: Who is up next, Neva?

DR. NEVA: No.

DR. BENENSON: Marsden?

DR. MARSDEN: No.

DR. BENENSON: Gunning?

CAPT. GUNNING: No.

DR. BENENSON: Farah?

DR. FARAH: No, sir.

DR. BENENSON: No argument.

MAJ. TAKAFUJI: The point should be made that these individuals who have been to Panama should be disqualified from donating blood anyway for three years since they have been in a malarious area.

COL. RUSSELL: That is a separate question. Let us not bring that up at 4 o'clock.

MAJ. TAKAFUJI: They are not eligible to donate blood. That is the policy we use at Ft. Bragg.

DR. BENENSON: Are there any other points to be taken up? From the military point of view, have we answered all your questions?

CAPT. BROWNLOW: Yes, sir, I think so.

DR. BENENSON: Anything else for us? I appreciate the time that the Committee has given to me and thank you very much and so on and so forth.

(Thereupon, at 4 p.m., the meeting was concluded.)