

1 And west Africa, you see lung cancer
2 winning, liver cancer quite low, and stomach
3 cancer not very low.

4 All the cancers in west Africa are
5 relatively low and this may -- some people say
6 this is an odd effect. And if you have stomach
7 cancer in west Africa, no one diagnoses you until
8 you get secondaries to your liver, and then they
9 call you liver cancer.

10 So the epidemiologic data doesn't always
11 support the link between H. pylori and gastric
12 cancer, and some people would say that's real.
13 Some people say it's side effect. So I think we
14 have a lot of -- long way to go and there's
15 definitely a lot of other factors, strictly
16 dietary factors which are important and maybe
17 explain half of the gastric cancers and the other
18 half might be H. pylori.

19 Thank you.

20 DR. FISHER: Thank you. Barry, the
21 study on the vitamin C and the CagA positivity,
22 was that controlled for amount of vitamin C intake

1 actually when they measured ascorbic acid in the
2 stomach?

3 DR. MARSHALL: I can't answer that for
4 sure. I'm sorry.

5 DR. FISHER: I guess because the other
6 question I'm coming in is, with the levels that
7 were seen, do you know how much -- in the gastric
8 juice, how much vitamin C you need to take to get
9 a sampling of ascorbic acid to that? And has
10 anybody looked at using vitamin C in changing CagA
11 status, or what vitamin --

12 DR. MARSHALL: Well, then again --

13 DR. FISHER: -- large doses of vitamin C
14 do to HP?

15 DR. MARSHALL: I don't see that you can
16 change CagA status because that's a gene that's
17 present in H. pylori and is quite stable.

18 But it was randomized that you had
19 people with gastritis and H. pylori and, you know,
20 some had CagA and some didn't. So that it's hard
21 to see how diet would affect that.

22 But I accept it as normal. It's

1 obviously not definitive data.

2 DR. FISHER: Has anybody looked at what
3 vitamin C does to HP?

4 DR. MARSHALL: No. But they've seen
5 what HP does to vitamin C and that there is data
6 from an acute infection by Dr. Savallah who caught
7 the H. pylori in the lab. And he had done these
8 gastric juice vitamin C labels. And he showed
9 that they dropped with the infection and then came
10 back up when he treated it.

11 So there is some other evidence
12 suggesting that it does bar it.

13 DR. FISHER: But he didn't go and take
14 megadoses of vitamin C at the same time --

15 DR. MARSHALL: No.

16 DR. FISHER: -- see what happens?

17 DR. MARSHALL: No.

18 DR. FISHER: Dr. Graham?

19 DR. GRAHAM: Yeah. But most of those
20 studies that you're asking about vitamin C have
21 been done. Actually they were done in the '30's
22 and '40's and '50's where vitamin C was a new

1 thing. And so they fed vitamin C and measured its
2 appearance in the body stores and the gut and
3 stomach and the urine, et cetera.

4 The problem with the whole vitamin C
5 story is that it's down in every form of gastritis
6 and in fact, it's down in all forms of
7 *Helicobacter pylori* gastritis, including duodenal
8 ulcer, which we know protects against gastric
9 cancer.

10 And so it's down in chemical gastritis.
11 It's down in pernicious anemia. And so it looks
12 to be -- to be interesting.

13 And all I could say, we like to study
14 easy things. But when you look across the board
15 and try and find something that is unique in
16 gastric cancer from other things, it doesn't come
17 up on that list.

18 The dissimilar -- there seems to be also
19 now that more and more studies are coming up to
20 show that the association of CagA with things is
21 also falling off and disappearing. And you know,
22 that's unfortunate.

1 But again, we only study the things that
2 we can see. And we've apparently mostly been
3 seeing things that are mirages.

4 DR. MARSHALL: Well, most of the
5 associations with gastric cancer being dietary,
6 just historically in the literature, and World
7 Health Organization has been doing all kinds of
8 studies around the world link -- trying to link
9 diet with gastric cancer risk.

10 And I can tell you that I would be very
11 disappointed. And even when they've measured them
12 in gastric juice and blood labels and all kinds of
13 dietary questionnaires, there's no real impressive
14 data coming up saying, "Ah, yes. Antioxidants are
15 great in prevention of cancer." And it's just not
16 a valid --

17 DR. FISHER: And nobody has looked at
18 vitamin E or Selenium and gastric juice and --

19 DR. MARSHALL: I think these data are
20 available from studies in Venezuela done by World
21 Health Organization and just being published this
22 year in different places. But it's very weak and

1 unimpressive. And not what you would hope for.

2 DR. GRAHAM: But the association with
3 fresh fruits and vegetables is almost uniform.

4 DR. MARSHALL: Yeah.

5 DR. GRAHAM: And the association was
6 made for fresh fruits and vegetables, meaning
7 vitamin C, and it might. But it probably doesn't.

8 And so it's our -- you know, it's our
9 approach to be reductionist, and now we will
10 probably have to be expansionists in that issue.

11 But I can't believe that you said half
12 of gastric cancer is not HP.

13 DR. MARSHALL: Well, the interesting
14 thing is that in the United States, the drop in
15 gastric cancer rate preceded the drop in H.
16 pylori.

17 DR. GRAHAM: Well, I agree with you. I
18 mean H. pylori alone is not the key variable. And
19 in fact, in the United States, except in certain
20 populations, we have a very difficult time finding
21 advanced intestinal metaplasia.

22 Even -- we just did a study in Corpus

1 Christi with looking into cross sectional and
2 bringing back the people with low pepsin engine
3 1's and pepsin engine 2's which tells you that
4 they have severe atrophy and endoscopying some of
5 them. And they did indeed have severe atrophy.
6 But they didn't have intestinal metaplasia.

7 And so there's something else that leads
8 to that step. And whether it's salt or diet or
9 different strains, it's not clearly going to be
10 CagA that makes that difference, but it may well
11 be a different as with ever bacteria.

12 I like to tell the family doctors, when
13 they say, "How does it cause all those things," we
14 know that streptococci can be asymptomatic or
15 pharyngitis or scarlet fever or rheumatic fever or
16 cellulitis, necrotizing fasciitis, et cetera, ecoli
17 with the same kind of approach. And I anticipate
18 that we may find organisms that are cancer --
19 cancerigenic versus ulcerogenic, but we haven't
20 figured out how to do that yet.

21 DR. MARSHALL: Dr. Graham mentioned the
22 association with fresh fruit and vegetables. But

1 the thing is when they tried to tease out what was
2 it in oranges, for instance, or fresh fruit that
3 made the difference, they didn't actually -- no
4 one has been successful in linking it to say it's
5 the carotene or the vitamin C or whatever it's
6 been, the negative --

7 DR. FISHER: Dr. Judson?

8 DR. JUDSON: The best estimates of the
9 experts present right now is that the attributable
10 fraction of gastric cancer caused by H. pylori is
11 what?

12 DR. MARSHALL: Well, in the U.S., it's
13 probably just about 50 percent. In the third
14 world, in Japan, it might be 75, 80 percent.

15 DR. GRAHAM: If you eliminate upper
16 cancer of the EG junction which apparently it
17 looks to be related to the absence of H. pylori, I
18 think that you would have a hard time pushing it
19 below -- really below 90 or 95 percent.

20 I mean these attributable risks are
21 based upon what the background risk is in the
22 population. And epidemiologists, you know, like

1 high relative risks or high odds ratios.

2 And to get a high odds ratio, you have
3 to have a disease that's vanishingly rare. And so
4 it's very difficult to get those.

5 But even in the -- you can make it get
6 bigger and bigger if you go into a younger and
7 younger age group like they did in Japan.

8 But without -- I mean we've known for 50
9 years that gastritis is a precursor of gastric
10 carcinoma. And Helicobacter pylori is a cause of
11 gastritis.

12 The other cause of gastritis, the
13 autoimmune, in areas where Helicobacter pylori is
14 rare, like around the Mayo Clinic, doesn't appear
15 to be associated with gastric carcinoma.

16 So I think that the data is -- when we
17 get rid of Helicobacter pylori cancers, the baby
18 and the bath water.

19 DR. MARSHALL: The nested case control
20 studies that he showed had attributable risk
21 around 60 percent.

22 DR. GRAHAM: But that's, you know, the

1 epidemiological problems.

2 DR. MARSHALL: The point is that if 100
3 percent of the population is H. pylori, you can
4 never link the -- you can never prove any risk at
5 all. And most of these studies show the link as
6 being in populations that have a very high
7 prevalence of H. pylori, so --

8 DR. FISHER: Next questioner, can you
9 identify yourself, please?

10 DR. ATHANIKAR: Nana Athanikar from
11 Josman Labs.

12 The vitamin C has an interesting story
13 though. This is not just a complete story because
14 it's not how much vitamin C that you ingest.

15 What happens after H. pylori infection
16 is that -- which is very elegantly shown by Dr.
17 Axon Cobler, that the mechanism by which the
18 vitamin C is actively secreted from the blood site
19 to the gastric lumen is shut off months and months
20 after the H. pylori infection has taken place.

21 So it is not how much you take orally.
22 In fact, the very neat thing, at 170 days after

1 the infection was the infection was almost gone,
2 the vitamin C levels were in the whatever -- in
3 the stomach were still near zero.

4 They injected 400 milligrams IV at that
5 time and look at the gastric juice vitamin C. It
6 doesn't come into the gastric juice.

7 So what has happened is, the mechanism
8 by which once the vitamin C is absorbed into the
9 system, it does not back into the gastric lumen
10 which in normal cases, comes out.

11 So the story is just not simple how much
12 is taken orally, but what happens to the vitamin C
13 secretory mechanism after the H. pylori infection.
14 I think that's the story.

15 And also the vitamin C stability at
16 higher pH is very low and higher the pH which
17 happens after H. pylori infection and repeated
18 gastritis, the even orally taken vitamin C may not
19 be quite stable because at higher, stability is
20 lower.

21 And also, an interesting thing is that
22 in developing countries where there is a higher

1 incidence of H. pylori gastritis, the percent of
2 those people developing gastric cancer is smaller.
3 Although developed countries it is smaller, the
4 percent of people that have H. pylori gastritis,
5 but greater percentage of them develop gastric
6 cancer.

7 What could be the story there? Because
8 of use of H2 blockers where the pH remains higher
9 and vitamin C stability is lower? Is that the
10 reason?

11 I don't know. The story is too
12 complicated, I guess. And the vitamin C
13 connection is more complicated than it appears on
14 the surface.

15 DR. FISHER: Thank you. Dr. Hopkins?

16 DR. HOPKINS: I'd like to ask a question
17 of Dr. Correa.

18 Does he have an opinion as to what point
19 in the stage of gastric cancer progression or what
20 histologic precursor lesion might become -- might
21 be irreversible through H. pylori eradication?

22 DR. CORREA: It's very hard to answer

1 and most people think that these incomplete or
2 colonic metaplasia is a real mild dysplasia that's
3 very hard to grasp. And -- but there are no good
4 data to show that.

5 The opinion is that the further along
6 the trail, the more difficult it is to regress.

7 DR. HOPKINS: So what patients would you
8 include in your trial?

9 DR. CORREA: Well, it depends on what
10 trial. We are conducting a trial in Colombia at
11 the present time where we are trying to see if our
12 intervention decreases the rate of progression
13 from atrophy and metaplasia to dysplasia. And
14 we're doing it six years totally. And having
15 findings at the beginning, at the middle, and
16 hopefully we get funded at the end.

17 But -- so that's -- so the research
18 trial at -- to see what happens with the
19 progression. I don't think we're ready to do it.
20 A cancer end point trial is very difficult.

21 DR. FISHER: Dr. Graham?

22 DR. GRAHAM: The problem in this country

1 and why it's so difficult to do a study in this
2 country is that first, very few people have
3 advanced lesions. And in long-term follow-up
4 studies even from Scandinavia show that in most
5 instances, you don't progress. The population
6 progresses at a relatively slow rate.

7 But in most instances, you don't seem to
8 progress, maybe one percent a year or less. And
9 so therefore, it would be very difficult to do the
10 study except in the countries where cancer risk is
11 quite high.

12 And in those instances where cancer risk
13 is quite high, it's interesting -- people don't
14 eat fresh fruits and bananas.

15 My rule of thumb, if you eat bananas,
16 you don't have cancer. If you eat bananas
17 regularly. And really kind of works well around
18 the world. And the people that eat those kind of
19 diets, don't have the cancers.

20 And if you go up in the mountains where
21 people don't get those, and then they get cancer.

22 DR. CORREA: I want to clarify a couple

1 of points.

2 In the first place, the ascorbic acid is
3 low in gastritis but not in all gastritis. It is
4 not low in duodenal ulcer.

5 And the second point is that you can
6 have a very high level of ascorbic acid in the
7 blood and still have very low ascorbic acid in the
8 gastric juice, as the doctor explained.

9 And, of course, most people, going back
10 to the attributable risk, most people have come to
11 around 60 percent as an attributable risk. Of
12 course, this is taking into account the
13 multi-factorial causation of the disease where you
14 can prevent the disease by Helicobacter or by
15 other means probably.

16 But if you -- the hypothesis is that if
17 you eliminate Helicobacter, the rate will come
18 down at least 60 percent.

19 DR. FISHER: Okay. We've got an issue
20 up here that I think is going to be sort of
21 difficult to discuss. We've been discussing it a
22 bit along the way.

1 The question being, "Please discuss
2 clinical study designs appropriate for the study
3 of non-ulcer related conditions, non-ulcer
4 dyspepsia, gastric cancer and lymphoma."

5 I think to discuss -- initially it was
6 mentioned as study end points for cancer and
7 lymphoma are going to be almost beyond the scope
8 of this committee to discuss, as far as
9 recommendations for the agency.

10 Dr. Hopkins, Dr. Fanning, comments about
11 that?

12 I'm not sure -- I mean we can spend the
13 whole day discussing -- weeks discussing this.
14 And we've got ten minutes before coffee break and
15 before people accuse me --

16 DR. LAINE: Shouldn't you separate out
17 lymphoma -- I mean the lymphoma is a separate and
18 pretty easy question I think for most people and I
19 think we should separate that out from the other
20 much more difficult questions.

21 DR. HOPKINS: I mean that's a good
22 comment, you know, MALT lymphoma has been shown to

1 be much more responsive. And one idea, I suppose,
2 would be to -- you know, if you can show
3 eradication in other patients, can you give a
4 claim from MALT lymphoma, even though these are
5 patients that are hard to find, and you know, you
6 may not find a study that is -- would we require a
7 study from MALT lymphoma before giving a claim?

8 DR. FISHER: I guess the question is are
9 you asking what are you claiming for?

10 Are you claiming for treatment of
11 lymphoma? Are you claiming for prevention of MALT
12 lymphoma? What are you claiming for?

13 I think if you were claiming for
14 treatment of it, then the study design becomes
15 that you need to have something more for treatment
16 of it and follow-up, in my opinion, in the
17 lymphoma, not just for eradication saying that
18 you've got a MALT lymphoma. You eradicate HP and
19 that's -- you can use it as a surrogate marker as
20 we've agreed upon for risk of recurrence of DU,
21 for treatment of lymphoma. I think that's a
22 different shtick.

1 I think if you're going for prevention
2 of MALT lymphoma, you've opened a whole basket of
3 things that can be -- then we should go back to
4 putting it into the drinking water.

5 DR. LAINE: MALT lymphoma is obviously
6 fairly rare. But I would think standard of care
7 right now in most people would add it on to the
8 NIH guidelines, along with ulcer disease, as a
9 treatment.

10 If you have low grade MALT lymphoma, I'm
11 not sure it's ever going to be a matter because I
12 doubt anybody is going to ever get a claim for it.
13 It's so uncommon.

14 But I mean standard of care now would be
15 if somebody has a low grade MALT lymphoma, you
16 would treat it. And as you know, there are clear
17 cases of it resolving.

18 I'm not sure how important an issue it
19 is here, but I think, you know, in terms of study
20 design, if you can prove that you can have a
21 certain number that resolved with treatment,
22 that's --

1 DR. FISHER: Well, again I think that's
2 a matter of --

3 DR. LAINE: -- it would be treatment,
4 not --

5 DR. FISHER: -- study design and what's
6 acceptable to practice perhaps in the literature,
7 as opposed to somebody coming forward for a claim.
8 I mean maybe we can ask for a show of hands from
9 the industry representatives in the audience.

10 Does anybody think they're going to want
11 to come forward with a claim for treatment of MALT
12 lymphoma in something that's going to be
13 prescription medication without -- under --

14 Okay. So there was one show of hand.

15 DR. MARSHALL: Two shows of hands. Same
16 company.

17 DR. FISHER: Barry, you've got a slide?
18 David?

19 DR. GRAHAM: Well, you know, the concept
20 that this is very rare is changing. And we see a
21 new case every three weeks, of our own, I mean not
22 being referred to us.

1 DR. LAINE: But those are normal
2 endoscopic findings, right, when --

3 DR. GRAHAM: Yes. Most of them are
4 asymptomatic with relatively normal endoscopic
5 findings whether they have a duodenal ulcer or
6 something else that we -- that we've been doing
7 all these years before.

8 And the answer is we were systematically
9 ignoring them, I guess. So I mean it will be a
10 problem in that there -- it's become so much more
11 common. But therefore, it's probably so much more
12 trivial and --

13 DR. FISHER: As opposed to somebody who
14 presents with what appears to be lymphoma and
15 they're endoscoped and found to have HP. And I
16 guess that's what I was thinking more of being if
17 anybody is going to want to have a claim for that,
18 as opposed to a finding that's found on endoscopy
19 when they're scoped, say for their DU or
20 something.

21 I'm not sure we need a whole big
22 discussion about it here to come to some sort of

1 guideline tonight. I think the idea about cancer,
2 we could again go through a whole thing of
3 epidemiologic studies.

4 I'm not sure that's something somebody
5 is going to ask for a claim. Well, maybe I'm
6 wrong -- ask for a claim of prevention of gastric
7 cancer.

8 But I think that maybe beyond the
9 purview of this half day series here --

10 Dr. Hopkins?

11 DR. HOPKINS: I don't know. I mean
12 we're hearing I think from the industry that some
13 sponsors would like to get -- I mean would like to
14 treat more patients than ulcer patients to make it
15 simple.

16 And I guess some would like to treat
17 chronic gastritis, which is essentially everyone
18 infected. And I don't know -- it hasn't been
19 completely clear to me from what the sponsors
20 want.

21 But the idea being that you would
22 prevent gastric cancer and so it gets back to the

1 labeling issue. Is, you know, can we actually
2 recommend in some sort of claim that you're going
3 to prevent gastric cancer by the treatment of
4 patients with histologic gastritis now that the
5 association between chronic Helicobacter gastritis
6 and gastric cancer is becoming more well
7 recognized, and can you do that without industry
8 data or do they need to generate data in some form
9 which would lead to some sort of claim for the
10 reduction of risk -- for reduction of gastric
11 cancer in certain -- in some patients, whether
12 they're high risk patients or whether they're
13 everyone?

14 I mean that's sort of the ideas that
15 have been pushed around in the agency.

16 DR. FISHER: Does anybody else around
17 the table want to comment on it? I don't want to
18 dominate the conversation.

19 Dr. Reller?

20 DR. RELLER: It seems to me -- I mean I
21 don't know whether industry wants or doesn't want
22 a claim for any of these things. But it seems to

1 me, if one wants to have a claim for treatment or
2 prevention of any of these distinct entities, that
3 one has to study them.

4 And the details of the numbers of
5 patients required, the objective end points that
6 were discussed, whether they exist or don't exist
7 and how they might be refined.

8 For example, with dyspepsia, there is
9 much discussion -- excellent discussion this
10 morning about that. But those are mechanics of
11 study design that could be addressed if there was
12 an intent to pursue a claim for treatment or
13 prevention of each of these entities.

14 But to do one and extrapolate to the
15 other based on what you think might be is -- it
16 seems to me unwarranted. It's -- I'm reminded of,
17 you know, some of the newspapers a couple of years
18 ago like, you know, eating bran flakes is probably
19 good for you.

20 But if you want to put on the box that
21 eating bran flakes prevents cancer, one should
22 have some data to put that on the box. And I

1 think that's where we are here.

2 If you want to label that one of these
3 drugs or any drugs or combination does something,
4 that there should be a study that shows that. No
5 matter how arduous it is, no matter how many years
6 of follow-up, one needs to show the appropriate
7 end point. And if a decision is made that it's
8 probably a good thing, that no one wants to invest
9 -- show it, then you have literature and
10 discussion and thoughts, but you don't have -- one
11 doesn't have a label that says it does something
12 that you haven't shown.

13 DR. FISHER: Dr. Kaiser?

14 DR. KAISER: Let me ask a question
15 though. I'm guessing that recent labeling on new
16 oral antibiotics about eradication of grouping
17 streptococcus -- streptococcal pharyngitis, they
18 are -- maybe it does include reference to
19 rheumatic fever. I'm quite sure that a new
20 antibiotic proof for eradication of streptococcal
21 pharyngitis would not have enough cases of
22 rheumatic fever to -- with reference.

1 So it has historical precedence for
2 that. So I'm -- we may have a situation that
3 indicates you could make a claim once it was
4 established about the causality of Helicobacter in
5 these various conditions.

6 DR. FISHER: Dr. Hopkins, you have a
7 comment?

8 DR. HOPKINS: Yes. To my knowledge,
9 only penicillin has that claim.

10 DR. KAISER: Yes, but -- but how about
11 in the labeling? It's only penicillin?

12 DR. HOPKINS: Right.

13 DR. KAISER: That doesn't --

14 DR. HOPKINS: Label or claim or --

15 DR. RELLER: I think, Allen, what you
16 would do is, I mean if you think it may have these
17 effects, but what you would put on the label is it
18 eradicates Helicobacter pylori, period.

19 DR. KAISER: Okay. So the penicillin
20 story is, we shoulder the penicillin and that new
21 drugs cannot carry the claim, only the
22 streptococcal, per se.

1 DR. HOPKINS: Dr. Reller's comments has
2 profound implications. I just emphasize that, you
3 know, traditionally we do not -- we require more
4 than must a microbiologic claim.

5 So when you only put --

6 DR. LAINE: Although I felt quite
7 comfortable with the surrogate marker of HP
8 eradication and ulcers is -- I'm not -- I think
9 the data are less strong about the intestinal
10 metaplasia.

11 I mean the question is I'm not sure I'd
12 be willing to accept the study to see what others
13 think where you just show that we treat with the
14 end point of intestinal metaplasia or chronic
15 gastritis or chronic atrophic gastritis.

16 And therefore if you are not willing to
17 accept that, doing these studies would be nearly
18 impossible, I would think. And as David Graham
19 said, really impossible in the United States.

20 So I would have a problem with those
21 surrogate end points myself.

22 DR. FISHER: Dr. Judson?

1 DR. JUDSON: I think I agree with these
2 comments. You wouldn't have permitted aspirin to
3 have a claim for preventing heart attacks just
4 based on laboratory studies of platelet
5 aggregation.

6 The studies of beta carotene and other
7 anti-oxidants had to be subjected to large
8 prospective epidemiologic studies. And I think
9 the same -- any time you have a rare complicated
10 condition like cancer, you have just got to avoid
11 getting on a slipper slope where people are
12 allowed to make their own extrapolations through
13 to the prevention of lung cancer, gastric cancer,
14 anything else.

15 It would appear that Japan would be the
16 obviously modern country with a sufficient rate to
17 carry out these studies -- carry out a prospective
18 study.

19 DR. FISHER: Dr. Graham?

20 DR. GRAHAM: And since the aspirin was
21 approved, though, what it does -- it had never
22 been studied which shows you that the system, you

1 know, does look at the laboratory data, as well as
2 the epidemiologic data.

3 One of our problems all of us have is
4 that -- particularly gastroenterologists, is that
5 we always thought about peptic ulcer disease as a
6 disease. And what we're stressing, at least when
7 I talk to the family doctors and the internists is
8 that they have to ignore the gastroenterologists.

9 And this is not a disease. It is no
10 more a disease than a fever blister. And do we
11 have an infection that occasionally causes these
12 problems and we have to think about it as an
13 infectious disease.

14 Unfortunately, you can't see it. But if
15 it were on the skin and you could see the skin
16 being destroyed chronically over time, I'm sure it
17 would not be pleasant to look at, and you knew
18 that one in six would get an ulcer that would
19 recur and continue to be a chronic problem the
20 rest of their life and one in a few thousand would
21 develop a cancer in that site, I mean you would
22 say, you know, this is not a good thing to have.

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1 But because we can't see it and we have
2 this historical background of gastroenterologists
3 making up a disease which is really a side effect
4 of an infectious process, you know, we have to
5 change our thinking.

6 And I hope that the next meeting, they
7 will exclude the gastroenterologists from the room
8 and so they won't -- they won't screw up the
9 thinking of the ID doctors which know how to deal'
10 with an infectious disease and it's various
11 ramifications.

12 DR. FISHER: Dr. Judson, do you want to
13 reply to that?

14 DR. JUDSON: I -- I think we're all in
15 favor of getting rid of this infection; that
16 nobody has shown that it's good for you or that it
17 exists in the long-term -- in the absence of any
18 pathology.

19 But I still think the issue of the claim
20 for cancer can be separated from that. People
21 will be attempting to eradicate HP with the view
22 that maybe it does reduce the rates of gastric

1 cancer.

2 But I think the FDA cannot make that
3 extra step and allow a claim for it. That may
4 have nothing to do with actual practice, but I
5 think it does have to do with their science based
6 integrity in these claims.

7 That's my view.

8 DR. FISHER: I agree with that. Dr.
9 Girardi, comment?

10 DR. GIRARDI: I've been very pleased
11 with the discussion this morning thus far, and I
12 think what -- I think we will take away from this
13 is that we don't really want to come to any
14 conclusions about, you know, particular specific
15 study designs with regard to non-ulcer dyspepsia
16 and gastric cancer because they are so difficult,
17 as we've heard from the elegant presentations this
18 morning, they're so difficult to study. And they
19 should be studied.

20 What we are going to take away is that I
21 believe that we have consensus over this
22 difficulty with our industry colleagues and that

1 what we need to do is to go forth in the future
2 and discuss the study designs based on our mutual
3 understanding of these difficulties.

4 DR. FISHER: Dr. Girardi, thank you very
5 much for leading us into coffee break.

6 With that, I'll break and we'll get back
7 here in 15 minutes, please.

8 (Whereupon, a break was had at
9 10:55 a.m. Until 11:15 a.m.)

10 DR. JUDSON: The second issue of the
11 day, I guess in a way we've organized this session
12 by starting off with the potential benefits of
13 therapy. Now we're going to, in a way, deal with
14 the other side, the potential risks of
15 antimicrobial -- widespread antimicrobial therapy.

16 And in this regard, Dr. Fanning will
17 begin the discussion of resistance implications of
18 widespread HP treatment.

19 DR. FANNING: Thank you, Dr. Judson. I
20 think my introduction was, in fact, going to be
21 just what Dr. Judson has said. But I have been
22 asked by several members in the audience in the

1 committee to begin by asking Dr. Correa why he's
2 been unsuccessful in treating his H. pylori and,
3 in fact, could that be because of resistance?

4 You don't have to answer that.

5 DR. FISHER: He wouldn't want to admit,
6 maybe compliance, right?

7 DR. FANNING: That's right. We will all
8 -- everyone will conjecture, but no one will ever
9 know.

10 So I think the other side of the
11 equation, that people have said, in principle, it
12 would be a very good idea to treat H. pylori, to
13 eradicate it in everyone and prevent all of the
14 things that it seems to be associated with,
15 particularly because there isn't an innocent state
16 of this organism, in that it is not known to
17 colonize, at least from the information we have.

18 So what is the downside of trying to
19 treat everyone? And the downside is the issue of
20 resistance.

21 I am not an expert in antibiotic
22 resistance, but there are a number of people on

1 the committee and probably in the audience who
2 know a good deal about this. So that when -- if
3 any of you have very specific questions, I will
4 not answer them. I will refer them to people with
5 greater expertise than I do.

6 But I will try to take you through a
7 simplistic understanding of the concepts of this.

8 Now we heard yesterday from Dr. Megraud
9 that there is specific -- there are specific
10 issues and there is, in fact, some data around
11 resistance of *H. pylori*. And this is an example
12 of some of the phenomenon that we'll talk about in
13 a broader sense.

14 There is already observed pretherapy
15 resistance to *H. pylori* in certain populations.
16 For example, he gave the example of Metronidazole
17 with 80 percent of *H. pylori* in developing
18 countries already resistant to Metronidazole, 50
19 percent in France.

20 And one can conjecture that there is a
21 lot of use of Metronidazole in developing
22 countries for treatment of parasitic infections,

1 and he gave the example that in France,
2 Metronidazole is used very widely in the treatment
3 of dental and oral infections.

4 In addition, the rate of H. pylori
5 resistance to Erythromycin was approximately eight
6 or nine percent prior to the notion of treating H.
7 pylori and stayed at a steady level throughout a
8 ten-year observation period.

9 So that here we see pre-therapy
10 resistance or what's called acquired resistance of
11 the organism already demonstrated, and this is due
12 to the use of antibiotics in the population for
13 other indications, not for treatment of H. pylori.

14 The second piece of information that Dr.
15 Megraud gave us had to do with post-therapy. And
16 he presented a number of studies looking at
17 development or at the resistance of organisms to
18 Clarithromycin after patients had received a
19 course of Clarithromycin in an attempt to
20 eradicate the infection.

21 And out of four studies, there was a
22 range between six percent and 47 percent, those

1 two being the outliers, with two studies about 16
2 and 17 percent in the middle.

3 How does this happen? How does
4 resistance emerge in the course of therapy?

5 Essentially -- well, there are probably
6 two mechanisms. One of them that makes a lot of
7 sense is that you have a population of organisms
8 that are not all the same, vis-a-vis resistance.
9 Many of them are susceptible, and you have a
10 couple who are kept at bay by this ecological
11 balance, who are resistant.

12 And by using a drug like Clarithromycin,
13 you eradicate the susceptible organisms and you
14 leave those ten that are not susceptible, and then
15 they tend to grow and to overgrow so that you now
16 have a population of resistant organisms.

17 The development of this is dependent in
18 part on how good your regimens are at eradicating
19 the infection because your population tends to not
20 necessarily be completely divided between
21 susceptible and resistance. But there are a
22 number of organisms in the middle who, if they got

1 enough antibiotic, might be eradicated. If they
2 got too little, might move towards the resistant
3 end.

4 And it's clearly also related to
5 compliance with the recommended course. If you
6 use too little antibiotic or you don't use it for
7 long enough for that particular infection, then
8 this phenomenon occurs of post-therapy resistance.

9 Now there are many complicated
10 mechanisms for all of this, and I'm not going to
11 touch on how this happens. And if people have
12 questions about this, there are people with
13 expertise who can answer those. But I will not
14 attempt to.

15 The other data that Dr. Megraud
16 presented had to do with -- what did I call this?
17 Okay. I've -- I'm getting my terminology mixed
18 up. We're getting into semantic discussions.

19 But -- okay, this actually is efficacy
20 and this was looking at a group of patients who
21 were treated with Clarithromycin.

22 If they started with resistance

1 organisms, the ones that, you know, were measured
2 according to the testing now, only 30 percent of
3 patients had a successful eradication.

4 Okay. If they started with organisms
5 that were deemed to be sensitive, only 83 percent
6 had successful eradication.

7 So we see that if you begin with a
8 population according to the susceptibility
9 measurements we have now, there is a difference of
10 efficacy of a regimen that's given.

11 Now ideally, you would like to see that
12 individuals who had Clarithromycin-resistance
13 mechanisms might have lower efficacy. Why should
14 the drug work in people who are presumably
15 resistant, and the answer is probably that we have
16 not refined our methodology for measuring
17 resistance quite as well as we need to and that
18 all sensitive organisms should respond.

19 And again the discrepancy in these
20 numbers is due to a lot of factors. Why do not
21 all organisms respond?

22 Perhaps because the regimen wasn't taken

1 appropriately or there's compliance or there's
2 absorption issues. There's a whole lot of things
3 that influence why effective regimens don't work.

4 But this raises the question, because
5 there is a discrepancy, if you know the
6 sensitivity of an organism in advance, you can
7 possibly predict a major difference in response to
8 treatment.

9 Should we be doing pre-treatment
10 susceptibility testing, and we hope that we'll get
11 into some discussions around that.

12 Now how does one prevent the development
13 of resistance of H. pylori through either of the
14 routes or through the routes we've talked about?
15 And Dr. Megraud had put up two possible
16 strategies, one of them being combination therapy,
17 which would allow you to treat those that are
18 susceptible to one -- to one antibiotic. And to
19 take that resistant population, treat them with
20 another antibiotic in order to get the whole
21 population down.

22 And the second is to try to minimize

1 failure of therapy, possibly by decreasing side
2 effects so that patients will take more drug or
3 other strategies.

4 Now that's a very specific focus on H.
5 pylori resistance. There's a much broader issue
6 that I'm concerned about in my current position,
7 and that is just the general concept of antibiotic
8 resistance.

9 What is it? Why is this happening? I
10 heard a very good presentation from someone who
11 went back into the history. We always go back
12 into the history of, you know, everything when we
13 are dealing with new problems. And it was really
14 quite significant to think how long the human race
15 has been around and to compare that to the
16 longevity of the population of microorganisms that
17 infectious disease specialists attend to. And I
18 won't even, you know, I won't even put the numbers
19 on the table because it's humbling.

20 We're very young, and bacteria,
21 particularly, are very adaptive and have been
22 around for centuries, and probably millennia. And

1 the development of antibiotic resistance is
2 probably an evolutionary bacterial adaptive
3 phenomenon that happens naturally in the course of
4 things.

5 There are a number of ways in which this
6 is induced. We've just had an example that you
7 can -- that it happens very specifically. That
8 you develop resistance of an organism you're
9 targeting for treatment such as the H. pylori
10 example. And this is through the selection of
11 resistant organisms, as I've said, and also
12 induction of resistance in organisms.

13 There is a second phenomenon because the
14 patient that you're treating for H. pylori isn't
15 sterile bacteriologically for other things and
16 has, in fact, a whole lot of other bacteria that
17 are part of normal flora.

18 And as you treat with antibiotics, you
19 in fact, select resistant organisms and can induce
20 resistance in organisms.

21 And this remains a community reservoir
22 for organisms that have been exposed for

1 antibiotics for other purposes, but that are
2 resistant.

3 And if you go back to my first slide,
4 what you will see is that we already have evidence
5 of that in the H. pylori example because in
6 patients who are treated for other indications in
7 some parts of the world, there is already evidence
8 that H. pylori that has not yet been treated is
9 already resistance to several antibiotics.

10 Well, I think the fundamental question
11 is antibiotic resistance as a public health
12 crisis. Is this real or is this imagined?

13 And having lived through the AIDS
14 phenomenon, which is my area of expertise, and
15 having, you know, suffered through people who, for
16 years and years said, "You're just overemphasizing
17 this and making it into a big problem," and then
18 ten years later, being drawn up to a commission
19 where they said, "Why didn't you do more back
20 then?"

21 I think we have to learn from the
22 examples of the past that are actually quite

1 recent, and really look at what is the data. What
2 is the potential and what are the consequences if
3 we don't act, and what can we do?

4 Now the truth is that antibiotic
5 resistance is indeed happening, and the people who
6 disagree over whether this is a real or imagined
7 problem disagree over the magnitude and the
8 potential magnitude of this in the future.

9 And as Dr. Megraud had said that today's
10 solutions are tomorrow's problems, I think
11 yesterday's solutions are today's problems around
12 antibiotic use in general.

13 We have a lot of examples. Tuberculosis
14 resistance is a huge problem in some parts of this
15 country. Gonorrhea has become increasingly
16 resistant. And in both of these situations, we
17 wind up -- and strep pneumonia as well, or
18 enterococcus -- we wind up with these megastrains
19 that are resistant to almost everything we have
20 available.

21 So that we are facing for the first time
22 on the heels of the euphoria of antibiotics

1 eradicating all sorts of infections and man
2 vanquishing some other species, we are maybe being
3 a little humbled in the sense that there is a
4 rebound here and that the bug population has come
5 up in arms and they're out to get us.

6 And we see development of resistance of
7 H. pylori as well. And why is this happening?

8 Well, the simple thing. Antibiotic
9 pressure. That's the concept that I -- I like
10 that term, you know. It sort of, without having
11 to go into great details, I understand that. You
12 know, it's like crowding pressure.

13 What does that mean? The more
14 antibiotic use we have, the more likely we are to
15 be pushing resistance to occur. And if one eases
16 up on that pressure, we may slow down a process
17 that may be inevitable.

18 Another concept that I think is
19 important, although I think we don't exactly know
20 how to do this entirely, is that now is the time
21 to institute steps to prevent further
22 magnification of this problem, not tomorrow when

1 we have the problem and we can't do anything about
2 it.

3 What are strategies to do this? Well,
4 one of the concepts is, you treat only when it's
5 necessary to treat. And I think related to the
6 discussion that occurred yesterday and today, the
7 question really to be put out is who should we be
8 treating?

9 We've heard that about 15 percent of
10 people who are H. pylori-infected will have
11 problems with ulcer, and that's one potential
12 population to target.

13 We've heard that everyone who has H.
14 pylori infection has some gastric inflammation and
15 that there may be some long-term consequences to
16 that.

17 Is that the group to target? We've also
18 heard in a very important way the number quoted
19 that half of the world is infected with H. pylori.
20 That's a lot of people. That's a big antibiotic
21 pressure.

22 Perhaps in the United States, that

1 number is smaller. Maybe it's in the order of 30
2 percent. I don't know.

3 We also know or at least without -- I
4 have been told that a recent article of the ACP
5 Observer has suggested that current therapy of H.
6 pylori in the United States is still relatively
7 low. That maybe about four percent of people who
8 have ulcers are being treated, not necessarily by
9 gastroenterologists, but if one looks at all
10 physicians.

11 So that we have relatively low therapy,
12 a huge population, and so we have potential
13 expansion of antibiotic use.

14 The other issue I think is that we have
15 a range of efficacy rates that suggests that some
16 people will need retreatment. Will they have to
17 be retreated once or twice or three times? And
18 when we start adding that up, again a lot of
19 antibiotic use.

20 So the question of who to treat is a
21 very, very important question and probably not one
22 that can be answered today, but one that we need

1 to start thinking about and working through.

2 And lastly -- and I think the important
3 thing, the people who are saying yes, antibiotic
4 resistance is a public health crisis, are not
5 saying, "Stop using antibiotics." What we are
6 saying is, use them appropriately. Use them only
7 when you need them, and use them well.

8 And so the second strategy is to
9 optimize your treatment strategy. And by that,
10 and it's always one has to juggle risk benefit,
11 all sorts of issues of this sort.

12 We look at efficacy, how good is the
13 treatment. Treat for as long as you need, but no
14 longer.

15 Make sure your patients take a full
16 course so that compliance and any issues around
17 compliance or why people aren't compliant should
18 be addressed.

19 The notion of combination therapy in
20 certain situations is important. In tuberculosis,
21 for example, perhaps in H. pylori as well, since
22 there appear to be fairly -- the organism seems to

1 have a rate of resistance -- of acquired
2 resistance that might warrant the use of
3 combination therapy.

4 And then lastly, do we need to do pre-
5 treatment susceptibility testing. And of course,
6 that is linked to how good is our testing and how
7 much do we believe that it will give us the
8 information we need.

9 So having given you a bit of a
10 framework, I'll stop here and return it back to
11 the Chair.

12 DR. JUDSON: Thank you very much. I
13 think we'll try to hold questions to keep on
14 schedule. We do have a full hour committed to
15 discussion in the afternoon.

16 And at this point, I would like to go on
17 to Dr. Linda Utrup's presentation on measuring
18 resistance.

19 DR. UTRUP: I am Dr. Linda Utrup and I
20 would like to speak to you about measuring
21 resistance today. I'd like to have you think
22 about four different topics as I go through this

1 talk.

2 One is the evidence of increasing
3 resistance with some H. pylori treatment regimens.
4 The second is a lack of standardization of
5 susceptibility testing methodologies. And the
6 third is the lack of universally accepted
7 procedural methods and the fact that there are no
8 standardized methods to assess three drugs for
9 synergy.

10 The topics I'm going to cover today are
11 mechanisms of resistance, resistance with H.
12 pylori treatment measurements, susceptibility
13 testing in general, and problems when testing H.
14 pylori, and problems when testing three or more
15 drugs with synergy.

16 My first topic will be mechanisms of
17 action and resistance in relation to
18 antimicrobials commonly used to treat H. pylori,
19 namely Metronidazole, Clarithromycin, Amoxicillin,
20 Tetracycline. Bismuth, I will defer to Dr.
21 Williamson from Glaxo who will be speaking next.

22 The only mechanism that is known for H.

1 Pylori at this time is the mechanism of resistance
2 for Clarithromycin. But often mechanisms are
3 similar among different genera of bacteria. And
4 discussion of these mechanisms will aid in the
5 understanding of the target sites and
6 dissemination of resistance.

7 This is a schematic of the bacteria cell
8 and here we have the cell wall. And this is made
9 up of peptidoglycan. And Amoxicillin acts by
10 inhibiting penicillin binding proteins which
11 inhibit the formation of the peptidoglycan.

12 Bacteria have developed beta-lactamase
13 that will break down the beta-lactim ring of
14 Amoxicillin, rendering the drug ineffective.
15 Beta-lactimases are carried most commonly on
16 plasmids, but they can also be carried on the
17 chromosome.

18 30 to 50 percent of H. pylori have
19 plasmids. Although there are no resistance to
20 Amoxicillin in H. pylori at the present time,
21 because they have these plasmids, there is the
22 potential for this resistance to occur.

1 Clarithromycin acts at the 50-S
2 ribosomal subunit. It blocks translocation,
3 polypeptide elongation and protein synthesis.

4 In H. pylori, the mechanism of
5 resistance for Clarithromycin is a point mutation
6 in the 50-S ribosomal subunit.

7 Tetracycline, another drug used to treat
8 H. pylori, reacts at the 30-S ribosomal subunit,
9 and it inhibits binding. Currently, there are no
10 known Tetracycline-resistant H. pylori strains.

11 Metronidazole, another drug for H.
12 pylori, oxidizes bacterial DNA, and this causes
13 breakdown of the DNA strands.

14 Resistance is linked to oxidation
15 reduction potential. And just how much resistance
16 do we have with Metronidazole with H. pylori?

17 Currently, it is approximately 25
18 percent in the United States. It is approaching
19 80 resistance in developing countries,
20 particularly in Africa. There is increased
21 resistance in women, presumably because they have
22 been treated with Metronidazole for gynecological

1 infections.

2 And there was a study done in Belgium
3 between and 1994 where they studied 20
4 pre-treatment H.

5 Pylori isolates each year and they found
6 that resistance increased from 30 percent to 80 --
7 48.3 percent between 1989 and 1994.

8 How much resistance occurs with
9 Clarithromycin?

10 Currently there is a low pre-treatment
11 level of resistance in the United States, but
12 there is evidence of induced resistance on
13 therapy.

14 In Belgium, that resistance increases
15 from .7 percent to 10.5 percent during that same
16 study that was carried out between 1989 and 1994.
17 There was also another study that was done that
18 investigated 57 patients that had duodenal or non-
19 ulcer dyspepsia. These patients were given low
20 doses of Metronidazole, Clarithromycin and
21 Omeprazole for one week.

22 At the end of therapy, 12 patients had

1 failed therapy. H. pylori was recovered from nine
2 of these patients.

3 Six of these nine patients had a
4 Clarithromycin resistance H. pylori isolates. Two
5 strains were resistance to Metronidazole and
6 Clarithromycin pre-treatment, and by the end of
7 therapy, there were six strains that were
8 resistant to Metronidazole and Clarithromycin.

9 Assuming that you believe that there is
10 resistance, the next question is should we
11 recommend susceptibility testing in H. pylori
12 clinical trials? And should we do this for
13 antimicrobials known to cause resistance, namely
14 Metronidazole and Clarithromycin?

15 Should we recommend it for
16 antimicrobials currently not known to cause
17 resistance, namely Amoxicillin and Tetracycline?

18 And if and when non-invasive methods are
19 approved, will we still require susceptibility
20 testing for H. pylori?

21 An important fact to consider when
22 answering this question is the fact that in

1 clinical trials, we do have failures, and what are
2 we going to attribute the failures to if we don't
3 have susceptibility testing to tell us whether the
4 organism is resistant or not?

5 Okay. Assuming that we agree we need to
6 do susceptibility testing, we got to the next
7 issue, and that is that there are no standardized
8 susceptibility testing methodologies for H. pylori
9 at this time. And this is a major problem. And I
10 have tried to initiate talking with the NCCLS, the
11 National Committee of Clinical Laboratory
12 Standards in establishing a committee to -- or a
13 group to look at these issues.

14 I talked with Dr. Megraud yesterday. He
15 is forming a similar committee in Europe. And he
16 said that he would like to collaborate with us.
17 And hopefully, we might be able to come up with an
18 internationally accepted standardized
19 susceptibility testing for H. pylori.

20 But in the meantime, I have NDAs I need
21 to address and we need to give some guidance to
22 pharmaceuticals companies as to -- for their new

1 studies that are coming up.

2 And what susceptibility testing regimens
3 should we suggest?

4 At the present time, we normally accept
5 broth macrodilution MICs, broth microdilution
6 MICs, agar diffusion MICs and disk diffusion for
7 new antimicrobials.

8 This is -- like I said, this is our
9 current one, things that we accept. There are a
10 number of people in this audience who probably are
11 not familiar with susceptibility testing. They
12 haven't done it yesterday. So I have been
13 requested to go over some of the procedures in
14 general of susceptibility testing.

15 For those of you who are quite familiar
16 with this, please bear with me for the interest of
17 updating some of our other colleagues.

18 The first one I would talk about is
19 broth macrodilution MICs.

20 In this method, we start out with a
21 series of tubes which have broth in it that has
22 been cad ion supplemented, and it has a pH of 7.2

1 to 7.4.

2 We dilute the antimicrobial in the tubes
3 in two-fold dilution, starting at, in this case,
4 168421 micrograms per mil and so forth down the
5 line.

6 The last tube here is our growth control
7 tube.

8 We take organisms and inoculate them
9 into tryptocase soy broth to a concentration of a
10 .5 McFarland, which is ten to the eight colony
11 forming units per amount, incubate it overnight at
12 35 degrees and read the next day for growth and no
13 growth.

14 Cloudiness in the tubes is growth and
15 clear tubes are inhibition of growth.

16 So in our first set of tubes here, we
17 have growth in all of the tubes. Therefore the
18 MIC would be greater than this last tube, greater
19 than 16 micrograms per mil.

20 And our second set of tubes, it is clear
21 here in the 16, 8 and 4 microgram tubes, but
22 cloudy at 2 and lesser concentrations.

1 And MIC is the least amount of
2 antimicrobial needed to inhibit the growth of the
3 organisms, so in this case, the MIC would be four
4 micrograms per mil.

5 The last set of tubes is clear for all
6 the tubes, so your MIC would be less than or equal
7 to .06 micrograms per mil.

8 It is very important when setting up
9 susceptibility testing that you test the correct
10 range of dilutions of your antimicrobial. If you
11 only test up to say .5 micrograms per mil and you
12 suspect that the susceptibility break point might
13 be less than or equal to eight micrograms per mil
14 and you have cloudiness in that .5 micrograms per
15 mil tube, you really are nowhere. You can't tell
16 whether you have susceptible, intermediate or
17 resistant isolate.

18 So generally you need to do several
19 dilutions around what you would expect your break
20 point to be and remember, of course, that you
21 don't really know what your break point is going
22 to be. You're trying to establish it.

1 So you might want to just take a range
2 from like .06 to 128 micrograms per mil to be
3 safe.

4 This method with all these tubes is
5 rather cumbersome and we have gone now more to
6 broth microdilution MIC's. And this method, we
7 again inoculate .5 McFarland concentration of
8 antimicrobial organisms into the tube. And in
9 this case, we're pouring it into an automatic
10 inoculator tray here. And this is the inoculator
11 that you will set down onto the tray and it will
12 automatically pull up the correct amount of
13 organisms so that in the end, you end up with five
14 times ten to the fifth colony formed units per
15 well in your microtitran tray.

16 This tray has ten -- or 12 different
17 antimicrobials in it ranging from the highest
18 concentration to the lowest concentration. Again
19 you would incubate this overnight, and the next
20 day, you would read in a manner similar to that of
21 your broth microdilution MIC's.

22 You would look for the least amount of

1 antimicrobial needed to inhibit growth of the
2 organism. And I know this might be difficult for
3 some people to see, but let's try to focus on well
4 number two here.

5 And the first three wells are clear.
6 And another common method that we use for
7 susceptibility testing is agar dilution MIC's.
8 And the major difference in this methodology is
9 that you're diluting the microbial into the agar,
10 rather than into broth.

11 You use what's called a stair's
12 replicator and we have this inoculating head where
13 we have put our different isolates into each one
14 of these wells.

15 You will have a ten to the seventh
16 inoculum that you put in there. And you have the
17 Sears replicator and on this side, you have your
18 agar plate with your particular concentration of
19 your antimicrobial and on this side where is your
20 isolates are.

21 And this head swings back and forth,
22 swings over here, drops in, picks up your

1 organisms. And then you drop it onto the plate
2 over here.

3 You incubate this overnight and then you
4 reap. And I think the same person that took the
5 purple bars on Dr. Barry Marshall's slide also did
6 my slides last night.

7 The basic idea here is that you look at
8 for your particular isolate on each one of your
9 plates, and again, you look for the lowest
10 concentration and impeding growth of your
11 organism.

12 Another methodology that we use is disk
13 diffusion. In this procedure again, we use our .5
14 McFarland and distribute it onto, in this case,
15 your Mueller Henton agar plate.

16 You dispense disks which have been
17 impregnated with your different antimicrobials.
18 Incubate overnight and then you read the next day
19 and you measure these zones of inhibition here and
20 you record in millimeters.

21 Disk diffusion testing does have some
22 issues when you're talking about H. pylori. For

1 one, it is a slow-growing organism and you wind up
2 having much diffusion of the antimicrobial before
3 you get growth in the organism. So you end up
4 with these very large zones.

5 This plate is 150 millimeters and some
6 of the zones for H. pylori can be up to 70
7 millimeters. So you can see, it's much larger
8 than what we typically see.

9 Also the NCCLS does not recommend
10 susceptibility testing for anaerobes which are
11 also slow-growing organisms.

12 After we have all this data, we plot it
13 with MIC's on the Y-axis and zone sizes on the
14 X-axis, plot our points and our aggression lines
15 to these. And assuming that these results
16 correlate with clinical outcome, we can establish
17 break points or MIC's here and for disk diffusion
18 here.

19 If you have organisms that don't fall
20 into these designated ranges, you could have spots
21 such as this where it is resistant to -- by disk
22 diffusion but susceptible by MIC's. So this would

1 be false resistant result.

2 If we don't accept zone sizes disk
3 diffusion for H. pylori, you might want to end up
4 plotting it such that you have MIC's on your
5 X-axis and the number of organisms on your Y-axis.
6 And it's possible that you might come up with a
7 five-motor distribution of these organisms.

8 And assuming that these organisms
9 correlate to clinical outcome, it would be easy to
10 draw your break point right in between these two
11 groups of organisms.

12 One of the questions that I am posing to
13 the committee is can we accept and give MIC's
14 break points in this type of a scenario even
15 though we don't have standardized susceptibility
16 testing criteria?

17 Another test that we heard about is the
18 E test for H. pylori. I would like to make sure
19 that I say that in clinical laboratories' routine
20 patients, you cannot -- E test is not approved for
21 testing of any drugs against H. pylori but it has
22 been used in investigational settings.

1 And E test methodology again you prepare
2 a plate similar to the disk diffusion plate, put
3 on your strip with your increasing concentration
4 of antimicrobial, incubate overnight, read your
5 MIC's where it intersects here on the strip.

6 So the question is, which susceptibility
7 testing methodology should we use for H. pylori?
8 Should we use agar or broth dilution MIC's?

9 Europeans, as you've heard from Dr.
10 Megraud yesterday, like the agar dilution method.
11 But I know there are investigators in this
12 audience who have been successful using broth
13 dilution MIC's.

14 Typically with regular organisms, we
15 accept either broth or agar dilution MIC's.

16 The next question is whether or not
17 we're going to accept disk diffusion and what
18 about the E test?

19 Okay. Assuming that we are going to
20 accept one of these susceptibility testing
21 methodologies, the next question is what
22 procedures, specific procedures should we

1 recommend? What should we use?

2 Should we use sheep blood or horse
3 blood? Should it be whole or late?

4 What amount of blood should we use?
5 Should it be five percent, seven percent, ten
6 percent?

7 What base medium should we use? Should
8 we use the typical Mueller Henton, or should we
9 use something that might grow the Helicobacter
10 better?

11 What pH should we use? Typically now we
12 test at 7.2 to 7.4. But the pH in the stomach is
13 acid.

14 What inoculum density should we use? As
15 I was saying in my presentation, we normally use
16 the .5 McFarland to start with.

17 A lot of H. pylori testing use up to a 3
18 McFarland. This is a quite increased amount of
19 organism.

20 What should be our incubation time and
21 what atmospheric condition should we test under?

22 What quality control organism will we

1 select for H. pylori?

2 So from this list there can be seen that
3 standardization of this methodology will
4 definitely be challenging, but I submit that it's
5 not going to be impossible.

6 Another challenging issue involved with
7 H. pylori is synergy testing when you have three
8 or more different antimicrobial -- or different
9 agents.

10 I'm not talking about synergy testing
11 now for every isolate in the chemical study before
12 the pharmaceutical companies have a fit here. I'm
13 just talking about maybe 20 isolates per clinical
14 study.

15 The way you do this methodology is you
16 dilute your drug B here and doubling two-fold
17 dilutions and you put it in the various wells
18 throughout the tray.

19 You also do your drug A in two-fold
20 dilutions across you tray in this fashion. Then
21 you would inoculate your organism, incubate, and
22 you would end up with -- could end up with a

1 result that looked like this, which is an additive
2 response.

3 There is not much effect of a
4 combination of the two drugs over that of either
5 drug alone. Or you might end up with a
6 synergistic response and where you have the effect
7 of A and B together, it's greater than either one
8 alone.

9 Or you could have an antagonistic effect
10 where the effect of A and B together is less than
11 either one of the agents alone.

12 So the issues are should we do
13 susceptibility testing in clinical trials and what
14 -- what should we do about inclusion of proton
15 pump inhibitors or H2 antagonists in this synergy
16 testing?

17 And as I showed you, that checkerboard
18 methodology was for two different antimicrobials.
19 What do we do when we have three or more different
20 antimicrobials though? What kind of procedure
21 would we even begin to use?

22 Now since I have a captive audience

1 here, I'd like to make some recommendations to the
2 pharmaceutical companies for some inclusions in
3 the microbiology portions of their NDA
4 submissions.

5 Please include culturing identification,
6 susceptibility testing and transportation
7 procedures in your NDA's. Please include as many
8 specifics on this as possible because as I was
9 saying, we're trying to establish these procedures
10 and you guys have done all this work. So you have
11 a good body of data that we could really use.

12 Susceptibility testing results for each
13 patient at all visits should be listed and they
14 should be correlated with clinical outcome. And
15 it would be very nice if this could be done on
16 some type of a summary table.

17 Remember that all components of the
18 double and triple treatment regimens should be
19 included in your NDA submission and in your
20 proposed package insert.

21 Now I know this runs very contrary to
22 what pharmaceutical companies want to do, but this

1 is combination therapy and we do have to deal with
2 all components.

3 And if there is failure on therapy,
4 please be sure to do susceptibility testing on
5 those isolates and specifically, if we can do
6 resistance mechanisms, please do that.

7 If there is anyone here in the audience
8 here who would like to help in the establishment
9 of susceptibility testing parameters, please see
10 me. I know there is a lot of expertise here. We
11 have two individuals who are already members on
12 the susceptibility subcommittee for the NCCLS, Dr.
13 William Craig and Dr. Barth Reller, our own blue
14 devil. And we have a lot of expertise in the
15 audience, too.

16 So anyone who is interested in helping
17 to contribute to that document, please let me
18 know.

19 So as a summary, I'd like to go back
20 over the points to ponder.

21 There is evidence of increasing
22 resistance with some H. pylori regimens. There is

1 a lack of standardized susceptibility testing
2 methodologies, and a lack of universally accepted
3 procedural methods.

4 There are no standardized methods for
5 assessing three drugs for synergy.

6 And as my last comment, we already have
7 dual and triple therapy for H. pylori and I would
8 like to propose that this therapy is only going to
9 get more complicated.

10 And I would like to propose what our
11 next generation of treatment for H. pylori will
12 be. And the caption on this says, "Take one of
13 these precisely every 10,187 seconds, and exactly
14 37 seconds after eating a bologna and cucumber
15 sandwich on rye bread.

16 "112 seconds later, drink 3.78 ounces of
17 buttermilk and eat three and one-half green
18 M&M's."

19 DR. JUDSON: Thanks very much. Again,
20 we'll hold questions.

21 Would Dr. Williamson be able to hold off
22 until after lunch at 1:00 break?

1 Thanks very much. We're through a
2 manipulation on schedule for lunch and we will
3 reconvene at one.

4 Thank you.

5 (Whereupon, a luncheon recess was
6 taken at 12:00 p.m.)

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A F T E R N O O N S E S S I O N

(1:15 p.m.)

DR. JUDSON: And our final two speakers have agreed to rearrange our order a little bit and to assure us that the presentations are largely complimentary. And I wanted to thank them for that.

Is Dr. Weissfeld -- there she is. And so we have a reversal order and she will be going first to speak on the microbiological aspects of *H. pylori*.

DR. WEISSFELD: Thank you, Dr. Judson.

DR. JUDSON: Do you have the technical support yet, or -- good.

DR. WEISSFELD: Yes. And I'm going to need the slide -- in order for you to see the slide, I'm going to probably need all the lights off because I'm going to show you pictures of *Helicobacter* actually on plates and they grow so puny that --

Okay. The first thing that I wanted to

1 do is actually share with you some of the
2 information on Helicobacter from those organisms
3 that are cultivable. And you heard yesterday that
4 there is one organism that has now been -- a
5 species has been added to the genus Helicobacter
6 but is not cultivable at this point. So these are
7 the ones that we can't culture.

8 And you see that the genus was actually
9 established in 1989 because these organisms were
10 formerly included in the genus camplobacter, and I
11 have included there the former camplobacter names.

12 And if you look at -- if you look at the
13 disease states caused by these Helicobacter
14 species, the first thing that I started to learn
15 about this -- these organisms that I looked at was
16 these organisms also are in the gastrointestinal
17 tract and I was concerned that we would be able to
18 differentiate Helicobacter fennelliae and
19 Helicobacter cinaedi from Helicobacter pylori.

20 But in fact, they really have very
21 little overlap even though both of them cause
22 gastrointestinal disease with Helicobacter pylori

1 causing gastroenterologist and duodenal ulcers and
2 then fennelliae and cinaedi causing bacteremia and
3 gastroenteritis in AIDS patients.

4 And actually when we see these organisms
5 in the clinical labs, fennelliae and cinaedi, they
6 are usually found from stool or blood.

7 One of the interesting points that
8 microbiologists can agree on in relationship to
9 the genus Helicobacter is the identification and
10 the identification is fairly easy as
11 identifications go in the lab because there are
12 basically four tests that you do: Gram stain,
13 oxidase, catalase and urease. And you can see
14 that that very easily separates out Helicobacter
15 pylori from the two other Helicobacter species
16 with Helicobacter pylori being urease-positive.

17 Now one of the interesting points that I
18 would like to share with you as far as
19 Helicobacter goes is that if you ask any of the
20 microbiologists who culture it what exactly to do,
21 everybody is going to give you a different answer.

22 And so as far as Dr. Utrup telling you

1 that there are no standard methodologies, that is
2 absolutely correct.

3 What I'd like to share with you today is
4 the expertise that we've developed after culturing
5 about 5,000 biopsies in various clinical trials.
6 This is by no means to tell you that we now
7 consider ourselves to be the experts, but this
8 system that we have worked out works for us, and
9 so if this can be considered, you know, that would'd
10 be fine.

11 But it's not to say that any other media
12 or methodology is any worse than what we have
13 developed ourselves.

14 One of the interesting things about
15 Helicobacter is that it's a microaerophile. And
16 what that means is, the concentration of oxygen
17 and CO2 is different in the environment than it
18 would be in ambient air.

19 The concentration of oxygen in ambient
20 air is about 21 percent. Helicobacter likes
21 oxygen but likes a lower oxygen tension of five to
22 seven percent.

1 And we've actually taken advantage of
2 that in designing a transport system that allows
3 the organism to be transported in reduced oxygen.

4 It also likes increased CO₂, between
5 five and ten percent, and in that, it's similar to
6 some of the more fastidious organisms like GC or
7 hemophilus.

8 One of the other things is it takes
9 about three or four days to grow, but we actually
10 hold our cultures for 14 days. And there are
11 significant numbers that grow actually between
12 seven and 14 days, particularly in the
13 post-treatment period. And it grows best between
14 35 and 37 degrees.

15 Now like the anaerobic organisms which
16 don't do very well on the bench top, this organism
17 will die fairly rapidly. So you have to actually
18 perform your testing in a very quick manner or not
19 open the various jars because some strains have
20 been shown to die actually within 45 minutes.

21 Okay. One of the things that I want you
22 to do, and I think we're going to need the lights

1 completely out for this, is to take you through if
2 some people haven't seen all the different things.

3 Can somebody get the lights, please?

4 Thank you.

5 This is the CLO test and if some of you
6 are gastroenterologists, I'm sure you're familiar
7 with this.

8 The actual organism is in the bottom
9 here and you can see that the CLO test, the biopsy
10 is actually inoculated into this little cupuole
11 and turns red if urease is actually released from
12 the organism in the biopsy. And the actual
13 negative CLO test is sort of a yellow color.

14 This is the transport system that we've
15 worked out, and I can tell you that one of the --
16 in some of the first trials that we worked on, we
17 did not use this system and I would like to tell
18 you a little bit about the system that we used in
19 early trials as opposed to this particular system.

20 In the earlier trials we did, we used
21 skim milk and actually I think you heard yesterday
22 that that and saline are two of the liquid media

1 that actually have been used.

2 But the problem with any -- using any
3 liquid media is that you have to actually get the
4 organism from the sites frozen. So that means
5 using dry ice.

6 And most of the sites hated having to
7 use the dry ice. It increased the transportation
8 costs dramatically because in some cases, we had
9 had same day delivery of the biopsies.

10 And so I looked in the literature and
11 there was a finished paper that was published as a
12 note in the Journal of Clinical Microbiology in
13 1992 that said that modified Stewart's agar, which
14 is actually the media that you find in culturette
15 swabs was good.

16 And so what we designed was a vial and
17 you can see the vial here with a little more agar
18 in it to produce a microaerophilic environment for
19 the organism.

20 And basically it's a clear medium that
21 just buffers the organism. And you would take the
22 biopsy and put the biopsy tissue right into this

1 piece of agar in there.

2 And then the interesting part and the
3 easy part about it is that you actually will
4 basically not have to use dry ice. And you can
5 transport this within 24 hours using just an ice
6 pack and a regular styrofoam box.

7 This has been very successful for us in
8 subsequent trials that we've done, and actually we
9 are running almost 99 percent correlation with
10 histology which again is different than what you
11 heard yesterday where culture was only 74 percent
12 and histology was about 94 percent sensitive.

13 To achieve a microaerophilic atmosphere,
14 and I didn't know how many of you were familiar
15 with this, we used jars that we used in the past
16 for anaerobic culture. And there are special
17 pouches that actually generate the right amount of
18 oxygen and CO2. And these are just two examples
19 of jar systems. And you can actually see the jars
20 here.

21 The pouches are also available. And the
22 problem with pouches for widespread trials is that

1 you could only fit two plates per pouch. And so
2 you could see that you would wind up with, you
3 know, many pouches all over the incubator.

4 So for large trials, both of them are
5 fine as far as the atmosphere. But for large
6 trials, you want to try to use the jars.

7 Now another advantage is the aero
8 systems usually have an indicator to show that the
9 system has achieved anaerobiosis. But
10 unfortunately for the amount of oxygen and CO2
11 that's in there, there is no indicator system.

12 And if you're going to quality control
13 the jars to make sure that the atmosphere has
14 reached the proper atmosphere for Hamplbacter,
15 what you're left doing is actually putting
16 Helicobacter in there with the actual tests that
17 you're setting up.

18 And that's what we do. But if you did
19 that in a pouch, you'd have basically one patient
20 specimen and one PC organism. So that's why we
21 went to the jar system.

22 Now if you look at this and you can see

1 the organism, you know, is growing here, this was
2 interesting to me because we have used very
3 successfully now for all of our biopsies Skeral's
4 media and blood agar. And the blood agar is on
5 this side and it's just five percent sheep blood
6 agar. Nothing special about it. Just the regular
7 media.

8 And then Skeral's media which is a
9 bacillar base with ten percent sheep blood. And
10 that medium is late.

11 And the organism grows very well on this
12 by plate on Skeral's. And yet in the sixth
13 edition of the Manual of Clinical Microbiology
14 which was published this year, they say the
15 Skeral's media is one of the worst for, you know,
16 isolating Helicobacter.

17 So again this is, you know, something
18 that with experience, I think, you know, we can
19 pull all the papers together and try to get to
20 some standardization of testing.

21 This is just a Gram stain of the
22 Helicobacter. One of the interesting things is,

1 for those of you who are familiar with the seagull
2 forms of camphlobacter genae, you can see that
3 while this organism is considered helical, you can
4 really see the helical shape much better in
5 pathology specimens than you can on Gram stains
6 from the actual colonies where the organisms can
7 look like strain Gram-negative rods.

8 Okay. And then for -- this is just an
9 example to show you of an oxidase test. This is
10 an oxidase-positive organism and you can see that
11 it has turned blue at the end of the strip where
12 it's been tested with the reagent.

13 And then for those of you who may not
14 have seen a catalase test, this is an example of
15 putting a drop of hydrogen peroxide on a
16 microscope slide and then using a wooden stick;
17 just touching the organism which is *Helicobacter*
18 *pylori* in this case.

19 All these pictures are of *Helicobacter*
20 and you can see how strongly catalase-positive the
21 organism is. This looks like something like
22 staphylococci, for example.

1 The other test is the test for urease
2 production. And this is just an example on the
3 left. You see a urease-negative organism and then
4 Helicobacter.

5 And if -- one of the interesting points
6 about this organism, it's one of only three
7 organisms, the other being Proteus and Brucella
8 that actually can turn this slant positive in less
9 than 30 seconds.

10 So by the time that you've actually
11 taken your colony off the plate and touched it to
12 the slant, the slant is already turning pink.

13 Now I'm not going to spend a lot of time
14 describing the susceptibility system because I
15 think Dr. Utrup did a very nice job doing that
16 right before this. But this is to show you some
17 of the susceptibility data that we have.

18 We have used in some of these studies
19 both agar dilution, Kirby Baller and BE test. And
20 my personal preference, as I'll show you, is for
21 the E test. I think it's the most reproducible
22 and the easiest to perform.

1 And actually in our hands, it has
2 correlated very well with agar dilution tests.
3 But I'd like to show you what the organism looks
4 like on all of the different media so you get some
5 feeling for some of the problems that we encounter
6 in the lab.

7 As Dr. Utrup showed you in her talk, the
8 Steer's replicator is the organism. It's actually
9 inoculated into these wells. And then in the next
10 picture, you actually see the technologists using
11 the inoculator head to inoculate what is a Mueller
12 Hentlin plate with ten percent sheep blood. And
13 that's the media that we prefer to grow this on.
14 And we do all of our susceptibility tests on this
15 particular agar.

16 And this is actually Helicobacter
17 growing here. And you can see how very faint the
18 organism actually looks when it actually grows up
19 on these agar dilution tests.

20 And just to reiterate, you have
21 different concentrations in the different agar
22 plates of the antimicrobial solutions that you're

1 working with and then you just spot inoculate onto
2 all the plates in order to get an MIC.

3 Now this is a Kirby Baller
4 susceptibility and it probably would have to be
5 pitch black completely for you to, you know, see
6 this. The reason being, if you remember what Dr.
7 Utrup said, that the zones are about 70
8 millimeters. That's probably right.

9 And you can see on the one,
10 Clarithromycin and Amoxycillin. If you get really
11 close to this, there's a haze right around the
12 edge where the organism is actually growing. The
13 rest of this is actually the zone of inhibition.

14 But on the Metronidazole, it's growing
15 in a little more. And while this organism is
16 still -- would be considered susceptible to
17 Metronidazole, although this is not a standardized
18 test and there are no known zone diameters; this
19 is what is written in the literature, you can see
20 a little better what you're dealing with in the
21 case of Metronidazole.

22 If you look at the E test strip, you're

1 going to have to wait until the next picture
2 because on the E test, you look as Dr. Utrup said,
3 for the point of intersection to get the MIC.

4 And in order to be able to see this, you
5 will notice that the next photograph is somewhat,
6 you know, out of -- not out of focus, but we'd use
7 a different lighting.

8 Here is a Metronidazole strip. The
9 Ampicillin and Clarithromycin strips that we used'
10 were both totally susceptible. So again, you're
11 only going to see growth right around the
12 periphery.

13 But you can't see the growth of the
14 organism right here. And basically what you need
15 to do is move the plates, when you're reading them
16 in the light, to see where the organism is
17 growing.

18 And, you know, that with some organisms
19 can be somewhat of a difficult task.

20 Basically for this we've tried several
21 things. It is better in the literature in which
22 Dr. Utrup alluded to and I'll share with you.

1 One is that the concentration, the
2 McFarland concentration that you start out with,
3 up to from a 0.5 to a four really makes no
4 difference. The results seem reproducible for
5 this organism no matter how heavy the inoculum is.

6 And .5 McFarland is about a ten to the
7 eighth. And a 4 McFarland is about a ten to the
8 ninth to a ten to the tenth.

9 So with a 2 log difference, you still
10 come up with the same results.

11 I found that it was interesting that in
12 replicate biopsy samples from the same patient,
13 this E test is very reproducible as far as giving
14 you the same MIC value, whereas some of the agar
15 dilution tests could have been off by as much as
16 two or three dilutions.

17 So for widespread clinical trials,
18 looking for MIC results, I think that this is not
19 a bad alternative to the agar dilution technique
20 at all.

21 Okay. And then I just wanted to show
22 you again a summary of the lab test for detection

1 of Helicobacter pylori which again I've taken from
2 the sixth edition of the Manual of Clinical
3 Microbiology. And basically you can see I did not
4 include the breath test since that is not approved
5 by the FDA at this point. So these are just the
6 approved tests that are available.

7 And I wanted to show you from a
8 compilation of the different papers that Bob Jeris
9 looked at when he wrote this chapter what the
10 sensitivity and specificity was since all of the
11 talks that have had to do with the microbiology
12 have dealt with how difficult it is to culture.

13 I feel that in the proper setting with
14 the proper transportation that culture actually
15 can be, you know, very, very sensitive now, as
16 sensitive in some cases as histology.

17 And actually our dealing with the CLO
18 test, from looking at results of CLO tests that
19 have been done offsite and comparing them to our
20 results show the CLO tests to be actually better
21 than we heard yesterday, than it was in Europe
22 where they didn't think it was a very good test.

1 But in fact, I think probably it's fairly
2 sensitive and, you know, there is no reason that
3 the gastroenterologist shouldn't feel confident
4 that, you know, they're doing fine when they use
5 it in most clinical patients.

6 Now obviously the reason to do culture
7 in the trials is to go ahead and look at the
8 susceptibility data. I don't think that
9 necessarily you would be treating your patients by
10 sending biopsies to the lab for culture. And I
11 don't mean to imply that. I think the CLO test
12 would do a very good job.

13 Serology. One of the drawbacks for
14 serology is that it is not useful in tracking
15 short-term therapy. So it probably does not have
16 a place in the clinical trials other than in the
17 pre-screen system.

18 Okay. Thank you very much.

19 DR. JUDSON: Thank you. And again if we
20 can just hold any technical questions for the
21 speakers until after Dr. Williamson has given his
22 talk.

1 Dr. Williamson.

2 DR. WILLIAMSON: Ladies and gentlemen, I
3 do want to thank you for allowing me to present a
4 talk on the issue of Bismuth products which may
5 prevent the emergence of resistant strains of
6 Helicobacter pylori.

7 I shall be talking in four discreet
8 areas in my talk this afternoon.

9 First of all, just briefly run over some
10 of the information in the literature that has been
11 presented both yesterday and this morning about
12 resistance acquisition in Helicobacter pylori.
13 And then just again briefly run through these
14 reduced efficacy against strains of Helicobacter
15 pylori previously resistant to antimicrobial
16 agents.

17 In the second to my talk, I want to talk
18 about the synergistic activity of ranitidine
19 bismuth citrates and various agents against
20 Helicobacter pylori.

21 And then lastly touch on the very
22 significant effect of Bismuth actually affecting

1 the acquisition of resistance within a population
2 of *Helicobacter pylori*.

3 Now some very nice studies published
4 five years ago by Haas' group in antimicrobial
5 agents and chemotherapy using in vitro selection
6 in the laboratory, it was quite clear that a
7 normally susceptible strain of *Helicobacter pylori*
8 was rendered resistant to either metronidazole,
9 erythromycin, tobramycin or the quinolones,
10 ciprofloxacin by using repeated subculture in the
11 presence of these selective agents.

12 However, in complete contrast, the
13 authors were completely unable to obtain any
14 strains resistant to bismuth, furazolidone, or
15 indeed, amoxicillin.

16 Our own experience with *Helicobacter*
17 *pylori* -- excuse me -- is based on growing the
18 organism for over eight and a half years now
19 within the company. We don't use the small glass
20 jars or the complete packs available in many other
21 countries, but we have actually invested in a
22 large microphilic chamber approximately the size

1 of this desk covered by this white cloth over
2 here. And within that, we can get several
3 thousand plates growing at a time.

4 Now I would like to show you -- this is
5 a picture given to me by Dr. Barry Marshall of a
6 single cell of *Helicobacter pylori* from an
7 individual being treated with Bismuth. The
8 organism is surrounded by mucus and you can see it
9 very, very clearly. The Bismuth is able to
10 penetrate the mucus and able to attack the
11 *Helicobacter pylori*.

12 Now I want to make a reference to the
13 fact that about 60 percent of strains of
14 *Helicobacter pylori* susceptible to eight
15 micrograms per mil of Bismuth, over 95 percent are
16 susceptible to 15 micrograms per mil.

17 And we have looked at over 70 strains
18 now from three countries in Europe, the U.S., Peru
19 and countries such as Thailand, China, Japan and
20 Africa. And we have not observed any what we
21 would call resistance to Bismuth.

22 I spoke to Francis Megraud yesterday.

1 Most of you would have seen that he's looked at
2 Clarithromycin resistance in France over the last
3 ten years or so and he's got a set of over 700
4 strains of Helicobacter pylori. He also has not
5 observed any strains resistant to Bismuth.

6 Now I noticed that a comment was
7 deferred to me this morning about the mode of
8 action of Bismuth. I would actually love to be
9 able to tell you what the mode of action.

10 However, it's quite clear from the
11 literature published a couple of years ago that
12 energy depletion within the organism must play a
13 key role in the gross inhibition activity of
14 Bismuth.

15 But my personal view is that the
16 activity must be linked to the organism's
17 metabolism. Certainly it's a captive file as this
18 thing pointed out. Absolutely requires carbon
19 dioxide to grow. Approximately 200 fold higher
20 concentration of carbon dioxide to get good growth
21 than you find in the normal atmosphere, of course,
22 combined with oxygen.

1 So there must be something about the way
2 in which the organism deals with electron flow,
3 which may well be inhibited by Bismuth itself.

4 Now in this slide I would like to
5 demonstrate that in contrast to Bismuth citrate
6 either with or without Ranitidine, you notice that
7 Ranitidine itself is not has slight activity
8 against *Helicobacter pylori*.

9 But when we start off with about ten to
10 the seventh -- that's ten million bacteria per
11 milliliter and expose them to Ranitidine Bismuth
12 Citrate, but only eight micrograms per mil, we get
13 complete killing of the organism over a 30 ton --
14 30 hour time period.

15 Just to reiterate that for the Bismuth
16 citrate on its own, although able to inhibit the
17 growth of *Helicobacter pylori*, certainly it does
18 not kill *Helicobacter pylori*. Ranitidine Bismuth
19 Citrate clearly kills *Helicobacter pylori*.

20 Resistance acquisition can clearly occur
21 previously to therapeutic to get rid of
22 *Helicobacter pylori* or during therapy to get rid

1 of Helicobacter pylori.

2 The best documented studies are actually
3 with Clarithromycin either as monotherapy or
4 combined with proton pump inhibitors. But it's
5 quite clear that in the organisms that fail to be
6 eradicated, quite a number of those become
7 resistant to Clarithromycin than it did to other
8 macrolides.

9 I would like to stress that the data is
10 very, very convincing, certainly with
11 Clarithromycin and other agents, but when we
12 combine the antimicrobial agent with an acid
13 suppressive agent, we certainly potentiate the
14 activity of that antimicrobial agent.

15 We certainly don't need achlorhydria to
16 do that. Either hyperchlorhydria or achlorhydria
17 is sufficient to potentiate the activity because
18 the key factor is to change the bulk pH of the
19 stomach to a pH which all of the Helicobacter
20 pylori are able to grow and therefore become
21 susceptible to antibiotics.

22 Now the data has been around for about

1 ten years now suggesting that strains that are
2 resistant to Metronidazole are far less
3 sufficiently killed by the so-called triple
4 therapies. You see here the range of efficacy if
5 the strain is previously susceptible to
6 Metronidazole ranges from 90 to 96 percent from
7 the relatively recent studies, whereas if the
8 strain was resistant prior to therapy with these
9 classical physical therapies the efficacy range
10 from 19 percent to 71 percent.

11 In all cases, less sufficient than with
12 the susceptible strains.

13 As was mentioned this morning by Dr.
14 Fanning, the recent results from Dr. Megraud's
15 associates in Bordeaux in France, Dr. Kaylor in
16 particular again highlight the fact that even if
17 you're using a non-Bismuth triple therapy proton
18 pump with Clarithromycin or Amoxicillin or with or
19 without Amoxicillin, it's very difficult from this
20 abstract to see exactly what the patients have
21 been treated with.

22 But nevertheless, if the strain is

1 resistant to Clarithromycin prior to therapy, your
2 chance for eradication is drastically less than if
3 the strain is susceptible at the beginning.

4 Now because we've seen Bismuth citrate
5 does contain Bismuth as an active moiety against
6 H. pylori, we actually suspected that we would get
7 synergy with various antibiotics which are
8 normally active in their own rights against
9 Helicobacter pylori.

10 Now in contrast to the, as it were, the
11 classical way of looking at the synergy between
12 antimicrobial agents which is where you look for
13 an increase of zone diameter size, so you look for
14 increased effectiveness in growth inhibition, we
15 looked at the far more tedious for more accurate
16 and here relative aspect of synergy with increased
17 killing of the organism.

18 Because clearly to get eradication of H.
19 pylori, we need to kill the organism as
20 effectively, and indeed as rapidly as possible.

21 In all of the viruses we tested, the
22 range of antibiotics from the major therapeutic

1 groups of antibiotics used for eradication therapy
2 and indeed found that Clarithromycin was best for
3 synergizing with Ranitidine citrate.

4 I would like to show you the results
5 that we found on a number of occasions with a
6 Clarithromycin resistant strain. Again we start
7 out with a high number of bacteria. From over
8 here, we've got almost a thousand million
9 bacteria. And when we've used a low concentration
10 of Bismuth here -- this is a quarter of the
11 concentration I showed you previously, we find
12 that there is no inhibition at all with Bismuth at
13 a quarter of this MIC, not surprisingly.

14 We've used Clarithromycin at 60
15 micrograms per mil and we start to see a side
16 effect. But when we put the two agents together
17 of these very, very low concentrations, we get
18 complete killing at some point between six hours
19 and 24 hours' exposure to those two agents, a very
20 significant synergistic activity.

21 Now I would just like to run through a
22 little bit more detail the application of the

1 well-used, two-dimensional checkerboard technique
2 to look for synergy between antibiotics.

3 Now the technique that's described -- as
4 demonstrated by Dr. Utrup this morning showed the
5 use of this two-dimensional checkerboard that's
6 looking for the effectiveness in terms of growth
7 inhibition of two agents when combined.

8 What we've done in our studies is to go
9 one step more and actually to look at how many
10 bacteria have survived or have been killed in the
11 presence of the two agents after a 24 hour time
12 frame.

13 And just to refresh your memories of
14 what we have done in this experimentation, we've
15 gone from a very high concentration of
16 Clarithromycin, 250 micrograms per mil. This goes
17 down in two-fold dilutions in this dimension.

18 Here we have zero concentrations, so no
19 agents whatsoever. This is the highest
20 concentration of Ranitidine Bismuth Citrate. This
21 is the highest concentration of Clarithromycin on
22 its own without Ranitidine Bismuth Citrate. And

1 that does not have Clarithromycin in that one.

2 So what we've done, we've incubated the
3 bacteria in the plate for 24 hours, and then using
4 an inoculation device taken with those bacteria,
5 inoculated them onto growth medium, incubated
6 those media for over seven or eight days to get
7 reculture of the organism, then evaluated the
8 results including the absence of antibiotic, we
9 get no killing of the organisms so the viability
10 is maximal. Two agents combined, we're getting
11 complete killing of the organisms.

12 On the previous graph where I had two
13 micrograms of Bismuth -- Ranitidine Bismuth
14 Citrate and 60 micrograms of it here. But you
15 will see this well here, for example, has only got
16 four micrograms to a mil of Ranitidine Bismuth
17 Citrate, but only eight micrograms per mil of
18 Clarithromycin.

19 We have developed a mouse model of
20 Helicobacter colonization and using suboptimal
21 doses of Ranitidine Bismuth Citrate and
22 Clarithromycin, four days of therapy, BD dosing

1 and evaluating them five days after the end of the
2 dosing, we got eradication in ten percent of the
3 mass in each of these cases. Subtle to more
4 dosing with these agents.

5 However, when we combine them of the
6 same dosage as shown here, we get a very
7 synergistic activity, a highly significant
8 difference.

9 Now the concept of synergy is very, very
10 nice because it has applications to the real
11 world. The real world is where we are starting to
12 observe strains that are resistant to
13 antimicrobial agents.

14 This is an organism isolated from west
15 London and it's normally susceptible to
16 Clarithromycin. And in vitro, following the same
17 sort of technology that Dr. Haas used, we selected
18 mutants that were resistant to Clarithromycin.
19 And this particular mutant is about 50 fold less
20 susceptible than the parental strain.

21 And here we have used low concentrations
22 of Ranitidine Bismuth Citrate and Clarithromycin

1 here at two micrograms per mil. So that's 30
2 times more than in the previous case, but again,
3 somewhere between eight and 24 hours, the
4 combination gives complete killing of the
5 organism.

6 When we look at a clinical isolate that
7 is resistant to Clarithromycin, we get exactly the
8 same situation. Now this is an organism that we
9 obtained from the University of Pedra, which is
10 about 60 miles from Bologna in mid-eastern Italy.

11 This is an organism that is a thousand
12 fold less susceptible to Clarithromycin than most
13 of the normal susceptible strains that you find in
14 the clinical situation.

15 And here, using a concentration of
16 Clarithromycin which seems relatively high, 31
17 micrograms per mil, and a relatively low
18 concentration of Bismuth, which on their own do
19 not kill, the combination again at some point
20 between 18 hours and 24 hours' exposure gives
21 total killing of this organism.

22 Now the reason I stress this fact --