MARKED A-CUT p. 8

Finklestein

?What would we find you doing on a typical day?

B Well, unfortunately too much of it (work) ~~is~~ at this desk, and I don't have enough time in the actual laboratory. This has been something that has evolved over the last five or six years. A I spend a great deal of my time in administrative activities either reviewing scientific papers for scientific ..for suitability for publication in scientific journals or on various and sundry kinds of committee work, both internal in the department and the school and nationally and internationally.

C My druthers would be to be across the hall ~~in the laboratory~~ at least checking more careufully on what's going on over there and..but fate has it that I don't have as much time for that as I would like. Actually, if you philosophize about it, probably it might be better because if I ~~did~~ do have any ability, it should be to dream up new things and get other people to do them and it's somewhat, one can consider it as, in part as a cop-out, that it's somewhat of a waste of my time to actually perform the tests if I can have somebody else do them.

?Researching on a broad scale?

Well, we have...

?Size of this laboratory reflects the.....?

Well, the size is relatively small, and the number of people who are in it is also relatively small for the output but I prefer it that way. But we have , we're interested in three major problems and a number of ramifications of each one. And, in fact, in some instances these are rather unpredictable,

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and we go where the action is. The major interest of the laboratory, ~~or my interest~~ has been the pathogenesis and immunology of cholera. Not that cholera is of any great importance in the U.S. but it does serve as a model for other enteric diseases, other diarrheal diseases about which we understand little, and which are causing a significant amount of morbidity and mortality in the world, not only in people but in reducing the available food supply by killing off domestic animals. So cholera is the model for these diseases.

We've been rather successful in recognizing and isolating and purifying the material that the cholera vibrio produces which causes the symptoms of cholera, that is, the cholera toxin, or choleragen, and this work has been exciting to many other laboratories abound the world, and especially so since the cholera toxin has been found to work by activating a very important host-cell enzyme called adenoid cyclase which leads to the production of what's called cyclic amp which is an important central metabolic regulator of almost all metabolic activities of man and other animals. So cholera toxin turns on this enzyme, cyclase, and it does it ubiquitously or promiscuously so that cholera toxin than has become the tool to people who have no interest whatsorever in cholera but are interested in cyclic amp mediated reactions and we furnished the toxin now to more than , since my secretary started keeping track, more than 250 different laboratories, most of which have no interest in cholera but are interested in cyclic amp mediated reactions in the central nervous system and the kidney and

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the liver and its effect, impact in immunological responsiveness, modulation of immune responses. Fingerprinting of malignant cells. The end is nowhere in sight , and I 've infact lost sight of the numbers of applications of cholera toxin in other studies. So one facet of our work deals with this fascinating protein and we've, as far as I see it, gone almost as far as we can go here in studying this protein although there are studies with which I am in collaboration which are being done elsewhere.

?What do you mean "gone almost as far as you can go here"?

The problems remaining are those of physical chemists and enzymologists who can deal more precisely with the x-ray crystallographic structure so that we know how this molecule is put together and therefore can begin to understand at that level how it works. We're into the molecular biologists can go into the cell and find out how it turns on adenalate cyclase. We only know operationally that it does, we don't know precisely how. And with this kind of understanding then comes control.

?Do you have any interchange with Dr. Fordtran?

Not as much as I'd like. We have never really mangaed to get together although his interests take over where ours leave off, and in the actual physiology of the gut and what cholera toxin does to it. We've never really arranged a suitable engagement or wedding.

The toxin shortly after its discovery was considered to be potentially an immunologic weapon against cholera. And this aspect has been extrapolated to actual field studies.

a cholera toxoid or inactivated toxin was made commercially and was tested in many thousands of people in Bangaledesh for its ability to protect against cholera. We don't have a good cholera vaccine, one that actually works in protecting against cholera. We have one that doesn't work so well. And the toxoid interestingly didn't work so well either, and it was in that study it was administered by the usual shot in the arm. And it has to be recognized that cholera is a disease that is restricted entirely to the lumen of the gut. The cholera organisms or vibrios reside on the surface. They never penetrate the gut, and their toxin is active strictly at the level of the most superficial cells of the gut lining the small bowel, so it's not so surprising then that attempts to produce immunity in this area by parenteral administration of a vaccine were realtively unsuccessful so that leads to the second area of our interest. It has been shown quite clearly that if a person has cholera and recovers, he is resistant to a second attack so the disease itself is an immunizing process. In the disease itself, as I mentioned, the vibrios grow in the lumen and on the surface, and they release their toxin which is quickly bound to the surface, and then with proper treatment the patient recovers and all this stops, and he gets rid of his vibrios, but he's had then an immunologic experience with both the vibrios and their toxin. And this experience leads to immunity. So we would like to develop a vaccine which would duplicate or imitate the natural process without producing the disease itself. We have..to this end we have isolated a strain of cholera which doesn't

produce toxin in significant amounts, and this mutant strain M-13 was fed to volunteers and it failed to cause cholera in any, and on 168 occasions in individuals that were fed massive 10 billion living organisms. When some of these volunteers were subsequently challenged with virulent cholera vibrois, they were found to be resistant, but unfortunately from one of the volunteers a strain was isolated which appeared to have regained the capability of producing cholera toxin. So although there were no untoward events, it appears that the strain is unstable. and therefore not suitable for larger scale studies. We're not too disappointed in this because this strain is not...we didn't consider it to be the ultimate. What we would like is a strain that produces cholera toxin which is not toxic, and this strain then..this mutant would stimulate both antibacterial and antitoxic immunity at the local level, in the gut. So this is one of our primary targets. And in order to do this, we have to develop methodolgy to increase our chances of recognizing such a mutant. We're dealing with populations of bacteria on the order of 100 million to 10 billion and how do we pick out the one or two mutants that we may obtain so this is the challenging problem at the moment in that area. Additionally with regard to cholera we recognize that in order for a cholera organism to cause cholera it has to have a means of stikcing in the gut. It has to recognize the small bowel of man and stick there in order to grow and produce its toxin. So we're now in a large-scale investigation of the adhesive properties of the cholera vibrios which enable them to

recognize their particular desired sited, and I think we've made particular progreess in this regard. We've isolated now a factor which blocks the adhesion of cholera vibrios to host cells and therefore this may be the factor that enables them to adhere because if it occupies the receptors sites, it seems like it may be relevant.

?Possible ways there?

So we hope that we can isolate ~~the~~ and characterize that material and learn how it works, again at the molecular level. Also this might be a suitable component of a vaccine to protect against cholera. The living vaccine that produces a toxin which is not toxic can be predicted to have even greater benefits than just the protection against cholera because it's becoming evident that a large number of the other diarrheal disease which are ~~invariably~~ and numerically and mortality-wise far more important than cholera work through a similar toxin, an immunologically related toxin, but if we can produce a satisfactory anti-toxic immunity in the gut against cholera, it should protect against the other diarrheal diseases and reduce the incidence, the world-wide incidence of diarrheal disease among tourists as well as populations in ghettos and in other developing areas, so those are our aims in cholera. We are also interested in isolating and characterizing a similar toxin produced by strains of E. coli, esch_____ coli, common resident of the gut of man, which also causes diarrheal disease and one of our occupations, that is the production and purification of this material, and on the third hand, we're also interested in , we ~~started~~ started

with studies on gonococcus, the causative agent of gonorrhea, which is a sexually transmitted disease, which there are more than a million new cases in the U.S. reported last year, and this year will probably be as good or better. And our studies with the gonococcus , attempting to understand something about the mechanisms by which it causes disease and what we can do about it immunologically have led us to finding that in order to produce disease in man the gonococcus has to have an ability to acquire its essential nutrients from man, this goes without saying, but in particular iron is a limiting factor. Iron in most animal hosts is unavailable to micro-organisms because it's tied up in complexes with host proteins so that the available iron is almost nil. And in order for a microbe to infect man it has to have a means of acquiring its required nutrients and especially iron which is available in very limited amounts, so we found this to be true with the gonococcus, we could stimulate, could make avirulent gonococci virulent by adding iron, and we could make virulent gonococci much less virulent by subtracting iron in a moedel system that we developed to investigate these aspects, namely a chick embryo model. When we extended these findings then to other organisms, other micor-organisms, other pathogenic micro-organisms, we find that we can't find any exception to the rule or to the hypothesis that the ability of a micro-organism to acquire iron may be teh single most significant attribute wichi determines not only the virulence or the ability to produce disease in man but also the nature of the disease

that is produced, in other words, those organisms that have only limited ability to acquire iron are generally restricted to surfaces of limited capability of invading human tissue or disseminating into human hosts whereas those organisms which have exalted abilities to acquire iron are the agents that cause disseminating, or septicemic type infections. ?Expect that research to be fairly _____?

I think this is a new world opening before us because some of the spinoffs that one can anticipate from such an hypothesis include the possibility of easily engineering living, attentuated vaccine strains (such as with a meningococcus on the basis of their inability to acquire iron, and we have done this. We have made meningococcal mutants which are, can't acquire iron and are avirulent and their virulence is truned on by adding iron. One would predict that these might be suitable strains to colonize the upper respiratory tract of man that won't be capable of invading, they'll stimulate local immunity, and they won't cause disease. Additionally, for example, if we can find out by what mechanism the gonococcus is able to acquire iron, we might be able to isolate the iron-scavenging molecule and make it antigenic, and it might be suitably immunogenic and we could use it as a vaccine against gonorrhea. So these are the three major areas--cholera, E. coli, and what started to be gonorrhea, but now extends into the role of iron in host-parasite interactions.

?Exictint enough to make you want to come to work?

I don't have any troubles about that. I'm on a 12-month

vacation every year. Don't tell the dean that. He might cut my pay.

?How do you feed this info to your colleagues?

It's a two-way street. People who come to me either a come as graduate students or come as post-docs, and we ste up a problem for them to work on, and thaey starte to work on that problem according to a fairly straight and narrow road and then they inevitably come to a fork in the road, and we then have to decide whether to go both ways at once or to take our chances on one blind alley rather than the other. And then as things go on, we come to another fork, and I get feedback from them about these problems, and we discuss them and see where to go. More often than not, the results that we anticipate are not the results that we get and sometimes this works out very well because our own expectations were usually more limited than what nature is trying to tell us. There's virtually no routine in my labs, fortunately or unfortunately we're doing something different almost every day.

?Real work analyzing results?

~~Probably~~ the real problems are in deciding what is most important, and most likely to give significant information. that will be broadly applicable. We turn many problems which are of interest by themselves but maybe it's better that somebody else solve them than that we spend our time on that.

?~~No shortage of problems~~?

There is no shortage of problems. The problem is too many problems.

?Any day better than another?

Maybe the 24th because I'll be back a day, and I can probably handle part of the things that have accumulated while I was gone. As far as I know.

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