

**AN ORAL HISTORY OF THE DIVISION OF CLINICAL PHARMACOLOGY
VANDERBILT UNIVERSITY SCHOOL OF MEDICINE
1963-2005**

The following is an oral history of the Division of Clinical Pharmacology obtained from John A. Oates M.D., Professor of Medicine and Pharmacology, and Grant R. Wilkinson Ph.D., Professor of Pharmacology. The transcript was made from over ten hours of taped interviews recorded on three separate occasions between 2002 and 2005. The work has been minimally edited and represents an accurate history of the Division from its inception in 1963 through 2005.

Professor John Oates' Oral History of the Division of Clinical Pharmacology recorded in the Spring of 2002 (April 23, 2002) and Winter of 2005 (January 21, 2005) at Vanderbilt University and the questioners in this history taking are Professor Grant Wilkinson of the Division of Clinical Pharmacology and Professor Jason Morrow of the Division of Clinical Pharmacology

GRW: Grant R. Wilkinson

JAO: John A. Oates

JDM: Jason D. Morrow

GRW: John, the art of therapeutics probably has a history of tens of thousands of years, since man first started eating plants and then that developed into the use of drugs, but clinical pharmacology is a more recent vintage, perhaps beginning early in the twentieth century. I wonder if you could give us some background of how you see the beginning of the history of clinical pharmacology. Where did it come from? What was the reasoning for it to develop as a scientific discipline?

JAO: I think there were a number of converging scientific endeavors that led to clinical pharmacology. They came to a head perhaps in World War II, during the course of the malaria program. With the war in the Pacific, obviously losses of troops to malaria was a major factor in the ability of the American forces, not only to survive but to be able to

fight, in that soldiers who were ill with malaria were removed from the front-line and out of commission. And there was a shortage of quinine during that time, particularly the supplies from the Far East were cut off, and a major emphasis was given both to understanding how best to use quinine and also the development of synthetic anti-malarial drugs. James Shannon, who was later to become the Director of the National Institutes of Health, was appointed as Director of the National Malaria Program and it had as its principal hospital base, the Goldwater Hospital in New York City, which was on an island in the East River, right across from the New York Hospital. At that time, it was fashionable, with questionable efficacy, to use malaria, or any kind of fever therapy in the treatment of central nervous system syphilis, accordingly, there was a paradigm for being able to infect in a very controlled way the malaria parasite into humans and then to assess the effect of drugs to halt it. So that was the investigational model that was employed at the Goldwater Hospital. Shannon, who had been a renal physiologist and worked with Homer Smith, assembled some of the brightest minds in the country, notably from New York, to participate in this endeavor, not only physicians but also a group of Ph.D. scientist that included Bernard Brodie or Steve, as he was called, as the chief person in that laboratory. They set out to perform what I think were the first clearly defined investigations that employed the relationship between the concentration of drug in plasma and the pharmacologic effect of drugs. Also, the studies of drug disposition or pharmacokinetics as it later became to be known. So, in Brodie's laboratory they developed analytical methods for measuring a number of these drugs using a fluorescence method for quinine. Sidney Udenfriend was one of the scientists that worked with Brodie in developing these assays and he, later at the National Institutes of Health, developed with Bowman the first Aminco Bowman spectrophotofluorometer; that was a scanning type of fluorometer which really opened up the use of fluorometric analysis. With these

studies, beginning with quinine they carried out investigations in which they first related the dose of the drug and the plasma level of the drug to its efficacy in halting malaria infection, and constructed very carefully the plasma concentration response relationships for the effects of quinine. They also began to examine the disposition of some of the synthetic drugs such as chloroquine. At the time, it was common to start an individual with malaria on what we would think of as a maintenance dose giving the same dose on a daily basis from the very beginning; the same dose that would be used in chronic treatment. Brodie and Shannon discovered that if a drug had such an extremely long half-life in the body then it took many days for the drug to accumulate in the body up to a therapeutic plasma level. Because of this they devised a loading dose of chloroquine that could bring the plasma levels up quickly, so that instead of having to bring troops out of the front lines and back to the field hospitals on the Pacific islands, where malaria was so severe, and hold them for a couple of weeks before the drug exerted its effect, they were able to use a loading dose, and within a couple of days have the patient effectively medicated for their malaria. Troops were then able to continue with their responsibilities. This was a revolution in the treatment of malaria, both in terms of morbidity and mortality of the infected troops, but also in terms of the efficiency of conducting a war in the Pacific. After the war Brodie received the Congressional Medal of Honor in which it was stated that his contributions probably did more to win the war in the Pacific than those of any General who participated in the campaign. This was very appropriate praise for this program in terms of its effect on the treatment of malaria. But it also really constituted the first systematic and strategically defined approach relating drug metabolism to pharmacologic effect. James Shannon summarized it shortly after the war in a Harvey lecture in which he essentially laid part of the foundation for clinical pharmacology. (Shannon, JA. 1945-1946. Re study of antimalarials and antimalarial

activity in the humans malarial, The Harvey Lecture, Series 41, 43-89) Another person who contributed to that effort indirectly but importantly, was E.K. Marshall, who was the Chairman of Pharmacology at Johns Hopkins. He had done work on the metabolism and plasma levels of sulfonamides, very important in World War II, and he and Shannon were in close touch during all of these wartime efforts. I think their thinking permeated the efforts of each other and I am sure Marshall's concepts about drug metabolism and plasma levels were very important in what Shannon and Brodie ultimately carried out. The Department of Pharmacology at Hopkins was called Pharmacology and Experimental Therapeutics and I think Marshall was very much a proponent of the philosophy that was implied by that title in that he believed that research in humans was very important in both drug development and the appropriate use of drugs in humans. So his work, independently, was important for the clinical pharmacology of those antibiotic drugs that also sprang up during the time of World War II. It was a different era. I understand that Shannon and Marshall would get together at their vacation cottages and sit on the front porch and discuss their approaches to this research.

So, with those achievements during the wartime effort, individuals were swept up into the post-war planning. Roosevelt commissioned Vannevar Bush to make a plan for this research in the health area for post-war era, and Roosevelt himself actually spoke at the construction of the first building at the NIH at the campus in Bethesda. He was also involved in the initiating effort, actually during the war, which led to the formation of the NIH. There were several directors at the NIH but the institution really took off when James Shannon became the director and he brought with him a number of people who had been in the Malaria Program, and Brodie was one of these. Brodie had one of the largest laboratories in what was then the National Heart Institute. He had two floors of

the building and among the people that he recruited was Sidney Udenfriend. Udenfriend became quite interested in serotonin metabolism and Albert Sjoerdsma, who was in the Experimental Therapeutics Branch of the Heart Institute, also had an interest. Sjoerdsma was an MD/PhD graduate and had been working on the pharmacology of serotonin. His entry into clinical pharmacology was an interesting one. He had been working on serotonin pharmacology using smooth muscle strips for the analysis of serotonin effects in the laboratory and one day as he was walking down the hall he was cornered by Robert Grant, who was one of the senior members of the Heart Institute. Bob Grant said “Al, you’re wasting your time back there in the laboratory. We have a myriad of problems about understanding the drugs that are used here in the clinical center on our patients and it’s just tremendously important for someone with your kind of background to apply pharmacologic principles to understanding how we use drugs and how drugs work in humans”. This really challenged Al Sjoerdsma and he made a key decision. He decided he would put aside all his non-human research that was going on at that time, and he would devote a year solely to clinical investigation to see how it went (Dr. Oates laughs), and whether it was something that he would like to be productive in. That was characteristic of Sjoerdsma to be able to take that kind of pivotal decision. So he created the section within the Experimental Therapeutics Branch that he engineered in such a way that it was a collaborative endeavor with Sidney Udenfriend. Udenfriend, by now, was getting to be fairly senior in Brodie’s laboratory and it became important to give him an independent laboratory of his own, so Udenfriend was made head of Clinical Biochemistry. I think Al Sjoerdsma had something to do with that and then the two of them set up a collaborative endeavor. Although there were two separate laboratories, they were scientifically linked and had conferences together and spent a lot of time planning their research together. Importantly, they shared the clinical associates who were

postdoctoral fellow equivalents. So that when I went to the NIH...

GRW: You started off though in medicine at Wake Forest, and then interned and did a residency at Cornell, but can you give that a little personal history...why did you go to the NIH?

JAO: Okay. Well, that started in my second year in medical school. I had taken a physiology/pharmacology course that the Chairman was very much committed to for training medical students in research...those of them that he thought were trainable (laughs). So, he gave us an option, either to take an exam or to do a research project. I, and three of my friends, got together and decided we would do a research project together. They gave us a project of investigating the effectiveness of peritoneal dialysis in the treatment of kidney failure. It was a blanket question, like, "You guys figure out how to test this". At the time, there had not been any human work on peritoneal dialysis and I think only an abstract had been published a departmental physiologist knew about....so they threw us on this project. We didn't have the resources to measure serum potassium, which we picked as an endpoint we wanted to follow for the animals in renal failure, so we took a surrogate measure of the electrocardiogram. We were able to show that with peritoneal dialysis of dogs that had their ureters tied off that we were able to keep them from developing the electrocardiographic parameters that were characteristic of hyperkalemia and they lived longer, as well. But in the course of that, I got very interested in the effect of potassium on the heart, something that is important to this Division, and as happens when you are a student, suddenly I was an expert in this area because I had read more about it than anybody else, I was asked to lecture the residents and really began to think about it and so I went to the Chief of Cardiology and told him I had some elective time free and also vacation time, and that I would like to work on potassium and the heart and

do research in the area. As a medical student, you know, I thought that the Chief of Cardiology could do anything related to the heart (laughs), so I naively went to him with this question and he being a very honest man who really valued the students said “John, I don’t know anything about research on potassium in the heart, so why don’t you go talk to the Chairman of Biochemistry”, who was Camilo Ardem a world renowned phospholipid biochemist from Italy. He had done the first work with radioactive isotopes tracing metabolic pathways in the world and came to the United States as a consequence of the outbreak of World War II. He had a substantial research enterprise so I went down and talked to Ardem and told him “I’d like to work in your laboratory on the effect of potassium on the heart”. Dr. Ardem, in his Italian accent, said “John, that is a very, very interesting project, but I think you should work on phospholipids”. (All laugh). This being the 1950's, I said “Yes, sir”, so he put me to work on phospholipids. I did a little piece of what ultimately got published in a JBC paper on the incorporation of unnatural amines into phospholipids in the rat and that got me interested in lipid chemistry. So when I began to apply for internships, I was attracted to the New York Hospital Cornell program, where the Chief of Medicine was David Barrow, who discovered the HDL influence in coronary artery disease and had a phospholipid laboratory. I, therefore, had this interest when I went to interview for my internship. Dr. Barrow, I think was somewhat taken by someone who might want to enter his program and was interested in lipid biochemistry, and he took this young man from North Carolina into the program; this was highly unusual at the time. While I was an intern I was scheduled to go into the Air Force for the doctorate draft or so-called Berry Plan, where after internship all physicians were drafted unless they had some other specific designation, which I did not. So I had my physical and my papers sitting on my desk to sign for my Air Force commission, however, some weeks earlier I had been sitting at the lunch room with one

of my resident friends, Dick Crout, who was a senior resident. He was talking about his experience at the NIH and I said “Dick, what is the NIH?”, and he told me and he had been a clinical associate there in the Cancer Institute and had now come back to finish his residency. It sounded very appealing to me to spend your time in the service doing research, and I knew that there was a phospholipid program in the Heart Institute, so I talked to my Chief, Dr. David Barrow, and said that I’d like to apply to the NIH. As was the case in those days, he got on the telephone to Luther Terry, clinical director for the Heart Institute, who was a friend of his. I didn’t hear the conversation, but I’m sure it went something like “Luther, I have a young man here who would like to come to the Heart Institute and I think you would like him” and Luther says “What’s his name? Okay. We’ll take him”. So, just as I was getting ready to go into the Air Force, I got a letter from the NIH saying that if I could arrange to have a year of residency after my internship, I could come to the Heart Institute as a clinical associate. Well, I didn’t have a year of residency. I was scheduled to leave the program and go into the Air Force and this was now getting into the spring and Dr. Barrow, the Chair of Medicine, had filled all the residency positions for the second year people. So, I went to Dr. Barrow and told him I had been selected, which he knew I would be, to the Heart Institute, and I asked him about a residency. He said, “Unfortunately, we have filled all the residency slots but let me see what we have here.” So he began flipping through his book. At the time, the subspecialty fellowships were called residencies and were run by the Chief of Medicine. So, as he flipped through he said “I have a vacancy here for the cardiac resident. Why don’t you do that?” I said “Dr. Barrow, I’m just an intern” and he said “John, I know that” (laughs). So, he took me from an internship to being what is equivalent to a cardiac fellow. This was not because of my great achievements, but because at that time with people going in and out of the service, it was very difficult to keep all the slots filled in a

rational way. So, I stepped into that breach and was a cardiac fellow for a year before I went to the Heart Institute. So, I went there with an interest in lipids. Rockefeller was across the street from Cornell and I had had a wee bit of contact with them in their work on free fatty acids and so took that with me when I went to apply at the Heart Institute. When I interviewed, we had a choice of which lab we wanted to work in. I talked to Don Frederickson, who was one of the chiefs in the lipid research area and told him I wanted to do something where I could learn biochemistry, but have clinical relationship to it. He said "We're not doing any clinical research, this is the wrong lab". So, I continued and interviewed with Al Sjoerdsma and the people in his group who were doing clinical research and were just having a great time with it. It was an exciting group of clinical associates working in the program and I was very taken with the idea to learn biochemistry from Udenfriend. So, I entered that program, instead of my prior intention of lipid research, and Sjoerdsma put me in Udenfriend's laboratory space. My office, my lab was right next to Udenfriend's office so he was in there supervising my work even though it was geared toward the clinical arena. So, that's how I happened to get to the NIH and I was in the Clinical Associate Program with Jean Wilson, Ned Hayber, Richard Crout, who became the Director of Bureau of Drugs for the FDA, and Leon Goldberg, who was head of Clinical Pharmacology at Emory and then Chicago.

While we were there, the Burroughs Wellcome Fund announced that they were sponsoring scholars in Clinical Pharmacology awards for a huge amount of money, I mean, it was \$15,000 a year, and they were awarding these to medical schools. We had never heard of Clinical Pharmacology and that was what Al Sjoerdsma called himself at the time, but we knew that it was close to what we were doing. So the four of us, Lou Gillespie, Dick Crout, Leon Goldberg, and myself got into the car one day and drove over

to Baltimore and talked to Lou Lasagna, who was chief of Clinical Pharmacology at Hopkins. He'd come out of E.K. Marshall's department, and taken the position of clinical pharmacologist at Hopkins, which now connected medicine with Marshall's department and he told us what he was doing and what he thought clinical pharmacology was. We came back with this new idea and Leon, who was senior to me, was quickly picked up by Emory to head their new clinical pharmacology program and he got a Burroughs Wellcome Award so the possibility emerged that there might be a discipline in this field.

The work I did at the NIH had to do with biochemistry of aromatic amines in humans and in the context of that, we participated in the discovery of the antihypertensive effects of methyldopa and the discovery that serotonin produced a psychological effects in humans and a number of other things. We did the clinical pharmacology of the first studies on the monoamine oxidase inhibitors, in which we developed dose-response curves characterizing the extent of blockade of amine oxidation in humans in the context of investigating those drugs as antihypertensives. So it was with that background and that type of research that was going on when Allan Bass called and Al Sjoerdsma put him in touch with me.

GRW: We were somewhere in the early 1960's and you indicated that Burroughs Wellcome was important in putting together funding opportunities in clinical pharmacology from which you can imply that there was a national sense that this was something that was needed and there was an absence of perhaps well-trained people, and that is why Emory and subsequently Vanderbilt, were recruiting. Can you give us a feel for that? I would suspect that the NIH, through NIGMS, was starting their big push to get clinical pharmacology as "a recognized area of endeavor".

JAO: That came about five years later. The Burroughs Wellcome was really, I think, on the leading edge of the field that was engendered by A. McGee Harvey or Mac Harvey, who was Chair of Medicine at Hopkins. He, too, had been connected with the Pharmacology Department at Hopkins with E. K. Marshall, and he had been sent for his postdoctoral work - he was interested in myasthenia gravis - to work with Sir Henry Dale in England. So, he worked with Dale on the pharmacology of cholinergic transmission and cholinesterase inhibitors, and returned to this country with this profound background in fundamental pharmacology and a keen appreciation, and, I guess, a sense of the culture of the British pharmacology community. He came back coupled with his background with Marshall with a very keen sense and vision for the importance of bringing the pharmacological sciences into a Department of Medicine. He was not insular, but that was his area and central to his thinking, so that from his very influential position at Hopkins, he contacted the chairman of the Burroughs Wellcome Fund, a company in this country and, of course, Sir Henry Dale was part of Burroughs Wellcome in England so he had that connection. Together Harvey and the chairman at that time conceived of the Clinical Pharmacology Scholars program and set this in motion at that time. That was well before the NIH later made the same sort of conceptual plan with Shannon playing an important leadership role along with Brodie and George Cosmides. Brodie and colleagues published their ideas in *Science* in the mid-60's (Brodie, BB, Cosmides, GJ, Rail, DP, 1965. Toxicology and the Biomedical Sciences, *Science*, 148:1547-1554) let's say about 5 years after Burroughs Wellcome had kicked off the idea. I don't know if they used the word *clinical pharmacology*, but they talked about the need for programs in the pharmacological sciences that were multidisciplinary in character and that brought the pharmacologic sciences to bear on drug development and understanding drug action and

drug metabolism in humans. That was an expression of what had grown out of the Malaria Program and also incorporated the concepts and practice at the NIH which brought basic and clinical disciplines together. It also brought in all the exciting things that were going on in Brodie's laboratory in drug metabolism and that some of his trainees were, in fact, clinical scientists even at that time. So they came together with this plan for programs in multidisciplinary programs in the pharmacological sciences and toxicology, and that formed the conceptual basis for what became the Centers Program. I think another influence was the thalidomide disaster which woke up both scientists and the country to the fact that the investigation of new drugs in humans was very shallow and did not bring to bear the kind of basic principles that would be useful in understanding drug toxicology and mechanisms and how to best use drugs in humans. So, there were convergence of things that led to both the NIH program and the Burroughs Wellcome program. It's probably...do you want to talk a little bit about what the NIH program was like that led up to methyldopa, because I think...

GRW: Yes, I think it would be interesting because, you implied earlier that you were put into Udenfriend's lab and there was this joint collaborative effort between him and Sjoerdsma's lab, and I see that over the years this interactive style has run through the Division.

JAO: That's right. Udenfriend was an extension of the Brodie conceptual approach to biochemical pharmacology, as it was called at the time. While I was there, I had the privilege of working with people like John Burns, Park Shore, and others in Brodie's laboratory. I didn't work directly with Brodie except one time he came in and borrowed some methyltyrosine from me to scoop us on some things! But the program was really

quite unique. Udenfriend had his own basic program and he was studying the aromatic L-aminodecarboxylase enzyme. He showed that it was a generalized enzyme that would metabolize all the aromatic amino acids, including tryptophan and 5-hydroxytryptophan and dopa. He also was working on the hydroxylation of tyrosine and phenylalanine and discovered the so-called NIH shift in which the proton moves in the context of the formation of a hydroxyl group on the phenyl ring. So he had a very basic program going but he was quite interested in clinical research, and I was developing methods for measuring the decarboxylase enzyme function and monamine oxidase function in humans using predominantly his fluorometric technologies. So, he was directing my work in analytical development and Sjoerdsma was directing the studies that we were applying to things like the monamine oxidase inhibitors. Thus each of the two scientists were doing their own things but they had this interface at which the clinical associates or postdoctoral fellows equivalents were working. Not too long after I got there, there was a big international conference on biosynthetic mechanisms for catecholamines and during that conference Udenfriend learned that people at Merck had synthesized this alpha methyl dihydroxyphenylalanine to assess its possible effect on the decarboxylase enzyme. It was made as a spin-off of the anti-cancer program in which they were making alpha methyl amino acids across the board to see if they could be useful in cancer treatment. But when they got to dopa, they saw that this might be an inhibitor of the decarboxylase. So they did the usual testing on it and found that it had essentially no pharmacologic activity in normal animals. The classic statement was made from one of the Merck pharmacologists, namely, that “we have given a gram per kilo intravenously and there is absolutely no cardiovascular effect”. That was because they were looking during a short period of time after the drug was administered and concluded that for a compound that had so little in the way of pharmacologic action it couldn't be a useful drug. Therefore it

“went on the shelf” at that point because of its negative profile. Well, Udenfriend was quite a persuasive and charismatic sort of guy and Carl Fister, who was head of the program that synthesized methyldopa, was still quite interested in the drug, accordingly when the two of them got together this created the opportunity for us to get some methyldopa for purposes of studying its effect on the decarboxylase pathway in humans with the understanding that it had no pharmacologic action; we were just interested in human decarboxylation and wanted to have a tool with which to evaluate it, along with its possible effect on catecholamine and serotonin biosynthesis. So Merck put it through the toxicology studies and sent it to us as an investigational tool really. Prior to its arrival we had planned to study it in people with pheochromocytoma and the carcinoid syndrome to look at effects on the catecholamine and serotonin biosynthesis, respectively. But it just so happened that when the drug arrived, Al Sjoerdsma was on sabbatical in Sweden and there were no patients in the hospital with these two diseases at the time. But we did have an active program looking at amine biosynthesis in patients and had developed the tools for measuring the decarboxylase activity in humans. So Lou Gillespie and I decided to shift the target and study it as an inhibitor of that pathway indirectly. A very key part of this was that we were conducting all of our studies on patients with hypertension. The patients in our unit on the clinical research center were all patients with hypertension because of the philosophy that anything we learned about amine metabolism could be of importance to developing better understanding of treatment of hypertension. So our normal volunteers, if you will, were not normal, but patients with hypertension. We did our studies on assessing conversion of tryptophan and tyrosine to their respective amines in patients with moderate hypertension, and using our endpoint of amine biosynthesis we were able to dose range until we got to a dose that finally shut off amine biosynthesis indicating that we were inhibiting the decarboxylase

enzyme. As we were looking at the numbers that were bouncing up and down on the chart, it looked like we had a trend with the blood pressure going down, so we were very clearly going home that night after looking at the medical chart and graphing out the averages of each day's blood pressure data, the data we had before, during, and after methyldopa. It was quite clear that we had a substantial reduction in blood pressure that returned to pretreatment levels after we stopped the drug. I was so excited that I called Lou Gillespie at home and he came over and looked at them and word spread around the Heart Institute, and the next morning at rounds Dr. Berletto who was the head of research at the Heart Institute came around on my rounds, looking over our shoulders at this. We then studied three patients and after that Max Tischler, who was head of research at Merck, flew down, looked at these three patients' data, had lunch with us, and said "I'm going to put this in pilot plant production so we can launch clinical trials on a major basis". At the time, there was really no good drug for treating severe hypertension. Guanethidine had just become available, but its use was a bit awkward and so Tischler appropriately got excited about it and based on three patients launched a pilot plant production in plans for clinical studies. So, I attributed all of that to the fact that this primarily took place in an environment where there was an emphasis on investigation in humans and in this particular circumstance humans with a disease, and that they were closely integrated with a relevant non-human biochemical pharmacology enterprise. So that was so productive, both for me and I think in terms of it's scientific output that when I began to look for a faculty position, I wanted an environment that could link fundamental sciences with clinical investigation, both in terms of the research but also the training because I think it's the training environment that was so important in the NIH structure..

GRW: So you came down to Vanderbilt to have a look-see and some ground work had already been done, I recall, by people such as David Rogers and Allan Bass. Thus the ground was fertile for that sort of endeavor. Do you recall some of that past history?

JAO: Sure. Allan Bass had...this gets back to Robert Grant at the Heart Institute again. Bob Grant had devised training grants in clinical pharmacology sponsored by the Heart Institute. He was the same one who got Sjoerdsma out of the laboratory and onto the wards and I thought he was a great visionary. He was an electrocardiographer, but he realized the importance of this field so he offered training grants in clinical pharmacology out of the Heart Institute specifically. So, when those announcements came out, Allan Bass obviously saw an opportunity there and it resonated with his own thoughts about what pharmacology ought to be. So Allan prepared a training grant in which he was the P.I., but the Director was to be appointed (laughs). He had a salary slot in there for the Director and he had laid out a general plan in which there would be training with the Pharmacology Department with their strengths in drug metabolism with Milton Bush and Jim Dingell. Jim had just come down from Brodie's lab as had Fridolin Sulser in psychopharmacology. And then on the medicine side, Grant Liddle and Elliot Newman and David Rogers as Chairman had put in their pieces of the application. At the time, Grant Liddle was very much a clinical pharmacologist in a broad sense. He was doing all the dose response studies on the mineralocorticoids that were being developed at the time; quite elegant work. So they put together a successful application and it was funded based on their plan and strategy. They had been recruiting for a good while before and had run several people through here before they finally put in a call to Al and asked him who was looking around. I think Al's group had gotten small for the two of us...(laughs)...his decision, not mine (all laugh). So, the time was right for me to take off.

I had been there for five years, with a one year break in which I went back to my residency. I did my second year of residency that I didn't get initially. (Laughs)

JDM: And so that was early in the 60's?

JAO: In 1963, I left to come to Vanderbilt. I had looked at several other places at that time. Good institutions, some of them, but what drew me to Vanderbilt, initially, was the strong commitment of Allan Bass in pharmacology and Rogers and Liddle in Medicine to truly develop an interdisciplinary program that linked pharmacology and other basic sciences to clinical investigation. Also, the CRC was here, which was an exact replica of the clinical site at the NIH. So the ingredients were here in which I felt I could build an environment to develop research and training in the area.

GRW: At that time, had the decision already been made or was it part of your development that, although the division was a joint one, physically it would be housed closer to Pharmacology than to Medicine? Was that an accident because Allan Bass was the P.I. of the grant or had a lot of thought gone into that...because it seems to be a critical step?

JAO: It was not an accident that he was the P.I. I think, you may recall, he had been Chief Resident in Medicine here and not only the usual involvement of a Chief Resident. It was during a war, so he was really almost "the" physician in charge of the medical service during the year that he was chief resident. All the doctors were gone, having gone to war, except a handful and he did all the CPCs. He did everything (laughs). So, he had been very much an internist before he delved back into pharmacology. He had the vision, like the name implied at Hopkins, of a having a pharmacology program that bridged into the

clinical sciences and he brought Liddle and Newman and Rogers in, and I was also attracted because David Rogers had been in the Department of Medicine at New York Hospital, Cornell. He had been the head of Infectious Disease there and so I knew him as a house officer in that program and that was certainly an attractive thing to bring me to Vanderbilt. I could clearly see that both Pharmacology *and* Medicine were willing to put something behind this endeavor. In most institutions in the country, at the time, it would be one or the other, but here Rogers, I think, wanted to make it happen as well as Allan. And, as to the space, I think, Allan and I both wanted to have this space close to the Pharmacology Department. However, space being the ultimate coin of the realm, initially we took it where we could get it and my first lab was in the VA...a long way from Pharmacology. Ultimately, I got a piece of space in Pharmacology and David Rogers plucked for me a laboratory that had fallen into disuse that had been a hematology lab and they weren't doing anything with it at the time. So, he gave me that, which was right around the corner from Pharmacology and so I had two labs then. It really all came together in the construction of the Werthan Building, when the fifth floor above the library in the old Medical Center went up it was possible to put all of our space together then in the Pharmacology Department. They even cut a corridor from that wing over into the fourth floor of Medical Center North and it was called the Oates corridor (laughs), It was a little covered area that went across the roof to the Department of Medicine offices (laughs) which reflected my keen desire to be close to Medicine even though my primary appointment at the time was in Pharmacology. I don't think anybody knew that, other than the Chairs and the Dean and me. My primary appointment was still in Pharmacology when I became Chair of Medicine.

GRW: I recall it was a very small, intimate, physical space, with everyone crossing and meeting.

JAO: There were two floors in Pharmacology and we occupied a piece of the fifth floor. And then when the Werthan Building went up, right adjacent to Pharmacology, the Dean, I think, then realized the value of the enterprise and was able to obtain a gift from the Upjohn Company that helped build the Werthan wing, or a piece of the Werthan wing for us to expand over into the wing above the Clinical Research Center. This gave us contiguous space, again, between Pharmacology and Medicine.

GRW: Part of winning the Dean over was success, when you went from just yourself up to a critical mass in a fairly short time. Can you tell us something about what your vision was and did you have such an ambitious plan that this would be the largest, most successful, clinical pharmacology program probably worldwide?

JAO: I don't think I ever conceived things in any sort of competitive nature. I was, at that time and I think still am, really turned on by asking questions and I think once you've been touched by discovery it really fires you. So, I was enjoying science and was able to make, I think, an impact in a small Department of Medicine. The house staff knew me. I was there at night, on weekends, involved in the care of their patients...you know, not always at the front line, like Tom Brittingham, but enough so that they knew about our program. I think I was excited about what I was doing and we had some successes and things happened so I, not being the type who's aspired to be number one, *per se*, but I sure did want to have the environment in which we could proceed with a research program and I knew that in order to do that we needed to have additional faculty and the kind of fellows that would make the program go.

JDM: When you came, were you identified as “the” Division, at that time? Can you tell us a little bit about how the recruitment went after you came or who envisioned the Division?

JAO: Well, the Division was initially myself which sounds unusual today, but at the time I think there were maybe a dozen faculty members in the Department of Medicine, full-time. I don't know how many there were on hard money for, but maybe four. The rest of the people were supported by extramural funds. John Flexner was supported by clinical income, but very little clinical income came to the Department and when David Rogers got us together when Medicare first came about, he brought home the reality that we were going to have to start billing patients for what we did. At that time, we didn't submit bills, and to impress things on us, he asked us, “How much do you think the whole Department of Medicine bills for in a year?” There were all kinds of wild guesses and it turned out to be \$12,000. Most of that was Flexner's money because – Grant, I and Elliott Newman - we weren't even thinking about billing anybody. That's just to indicate what faculty support was at the time. So, there were divisions of one or two or at the most three people. Grant's activities were largely through his fellows. We began to recruit when we got the Burroughs Wellcome money; that opened the way to recruit Alan Nies, for example, and the Center, of course, allowed us to recruit both in the basic area and to support clinical faculty that allowed bringing David Shand. Bill Pettinger, I guess was the first person who came on to the faculty. Bill had been at the NIH, a resident at Yale, and then came here for a further fellowship. His arrival was, I think, very well timed in that we were just getting into the guanethidine-adrenergic neuron-norepinephrine uptake mechanism. He had done a lot of work with tyramine infusions in humans to test indirectly-acting amines so we were able to apply that to our studies on tricyclic antidepressants, and that was part of the Center grant initial application. We had recently

had publications in JPET and JCI on the guanethidine mechanism and Bill played a role in that along with one of Vanderbilt's two first MD/PhD students. One of these was Jerry Mitchell, who worked with me, both on some of the clinical studies and on the basic work on interaction of tricyclic antidepressants with guanethidine that led to the discovery that guanethidine was taken up into the adrenergic neuron by the norepinephrine pump. And that, for those who are not familiar with that story, also got started in the clinics. I and a resident were seeing a patient, who had been referred here with uncontrolled hypertension. The resident had worked the patient up and came out and shook his head and said this patient sure is on a lot of drugs. And the patient was on a **huge** dose of guanethidine, much bigger than is usually required to control blood pressure, which was still humongously out of control; the drug wasn't touching her. So, when he called my attention to the number of drugs, I looked over the list and saw that one of them was a tricyclic antidepressant and had the intuition that maybe this drug, as I knew that it affected amine uptake, might be affecting guanethidine, although I had no clear notion about why it might be, and I didn't know that it affected amine metabolism. So, we stopped that, and about a week later, I had a call that the patient was unable to stand because the blood pressure was so low on the same dose of guanethidine. I think it was clear that she'd had an interaction there and when we took away the tricyclic, guanethidine became excessively effective. So, then since it was just anecdotal, we set up some studies at the VA to evaluate the effect of tricyclics on guanethidine effect on blood pressure in a controlled fashion and further studies confirmed the anecdote leading us to ask why. Jerry, who had been part of the clinical study, then picked up in the laboratory and using uptake into heart slices, we were able to show guanethidine's selective uptake by the norepinephrine pump. So, that was the project that started with a clinical observation developed with a fundamental observation and led to a mechanism.

GRW: Now, Bill Pettiger was here and then John Cavanaugh was about the same time?

JAO: John Cavanaugh was a fellow and he was an MD/PhD from Iowa who worked in drug metabolism and then David Shand, who was more experienced clinically and also had a PhD in pharmacology, arrived. I guess we started two projects at that time. One of them was with a drug SU13197. That started because my work on guanethidine required, or led me to want to get a guanethidine tracer, which Grant remembers when he first came, so that we could look at guanethidine pharmacokinetics in humans and try to show that in humans you could block guanethidine uptake in the deep pool. I went to England and got the English branch of Ciba-Geigy to make us the radiolabeled guanethidine tracer. In the course of presenting that work to the people in the US with Ciba, Gil McMann, who was head of clinical research at Ciba at the time said “We’ve got a drug, an antiarrhythmic drug that we ought to learn more about its metabolism”. I had told him about our commitment to drug metabolism investigation. So I told him we would look at it if they would make us a tracer and they did, and this drug was SU13197, which was a fairly potent antiarrhythmic drug that had been tried in a few studies in humans with arrhythmia in Europe. It seemed to be very effective, but occasional patients got an excessive effect of it and it was clear that they didn’t have a clear handle on how to use the drug. So, this variability led them to want us to evaluate it for them and we set the study up so that we did both an intravenous radiolabeled drug and an oral drug separately and evaluated the kinetics of each. David Shand and Jack Cavanaugh were both involved in that study, and I remember David Shand and me sitting in my office cogitating over this data. When we gave the drug by mouth, very little of it was getting into the circulation as unchanged drug, as opposed to when we gave it intravenously. We also had the urinary and the fecal

radiolabeled metabolites so we had the complete metabolic picture and knew it wasn't failure of the absorption because tracer was getting in and tracer was getting out in the urine, but not active drug. At the time, the pharmacokineticists described everything by mammillary models as they called them at the time; the idea that something would be cleared in the first pass wasn't part of that model. So, with those studies we formulated the ideal of a first-pass clearance by the liver and gut and found that in this particular drug it was fairly high. I think, probably the most important thing was that it was variable between individuals which I think, is a general principle of low bioavailability in that it is also variable. So, it became clear for that drug that with this low bioavailability due to extensive first pass metabolism that the variability was too great for it to be a safe drug. We went on to do some clinical studies and also found that occasional patients got proarrhythmic affects that we didn't like and we thought we knew the reasons for them. So, right on the heels of that, another clinical lead developed and that was pediatric cardiologists called us up and asked us what is the dose of propranolol for a child. They had a child with subaortic stenosis IHSS and they had just catheterized the child and demonstrated this and didn't know how much to give of the drug because it was brand new and no work had been done in children. So, David and I sat down and thought about this and decided well, the only way to do this was to take the human dose which was known from some previous work and determine the plasma level in humans and then get a dose for this child, who was, I think, less than a year old, and find out what dose in the child would be required to achieve the same plasma levels. So, to address this clinical problem, we ended up doing pharmacokinetic studies on propranolol in adult human volunteers on which to base this child's dosage. In the course of that work, which came right on the heels of the SU13197, it was also apparent that there was a big first-pass metabolism of propranolol as well and that led to a whole series of studies that Grant,

David, Alan Nies, and Bob Branch participated in very productively.

JDM: Maybe we should back up and go back to the issues related to the original Center grant in the mid-1960's and what led up to that and what transpired.

JAO: I mentioned, previously, that in the article published in *Science* which established the conceptual basis for centers in pharmacology and drug toxicology that would address the need to bring fundamental pharmacological sciences to bear on understanding human drug action and metabolism. It had a very strong emphasis on biology, this was before molecular pharmacology, and it referred to biochemical pharmacology, which was what Brodie's laboratory was called. I recall that it involved not only drug metabolism with Jim Gillette and Brodie and others, but also involved biochemical mechanisms and serotonin metabolism, catecholamine metabolism, and histamine metabolism, that were underway big time at the NIH. The value of those studies had become apparent, so that led to decisions at NIGMS to think about funding centers. And although I wasn't a participant in the early conversations, the NIGMS staff knew about what was going on at Vanderbilt and initially proposed creating a Clinical Pharmacology/Drug Toxicology Institute off campus in a building that they would fund to build. It would include the biochemistry and toxicology program that Bob Neal then headed, but now Fred Guengerich leads, and it would include our clinical pharmacology group with the allied activities in drug metabolism in pharmacology. Allan Bass and someone from Biochemistry, I guess it was Bill Darby, had discussions with them and they got to a point where they had a rough plan of a huge institute. I heard the figure \$30 million tossed around for how much they were going to put into building this structure and providing staff and so on. I told Allan that it was not appealing to me to be extricated

from the Medical Center, taken away from the Department of Medicine, with its clinical activities and set somewhere else off campus. I thought that defeated the whole purpose of what we were about because I thought involvement in clinical investigation was key to the clinical problems in medicine and I could see isolation rearing its ugly head if we moved off into such a center or such an institute, as they were going to call it. So, I told Allan I was unenthusiastic about it. I wanted the money and he wanted the money and I think he had great ambitions; I know he had an ambition for us to succeed in that area, so he told the NIH that its particular formulation of an institute wasn't quite right for us and that we wanted to be involved in the medical school. So again, second-hand, I understand that they said "Tell us what you want, give us a proposal", so that led to our Center grant proposal at that time. In that, our whole intent was to put this program at the heart of Pharmacology in the Medical School environment, and it sold. I don't know all the people who were on the first site visit, but I remember Jim Gillette, who was a leading individual and John Burns, who by then had become head of research at Roche, but had been in Brodie's laboratory and they were both part of the evaluation group. They were both very focused on drug metabolism and, I'll say that unlike some of the scientists that we deal with on Study Sections today, these guys were terribly excited to see an opportunity for their basic finding on drug metabolism now to be exploited in clinical studies. So, the idea that there would be translational research, that would build on what they were doing, was supported passionately. They were strong advocates, I mean they were scientifically critical, as they should be, but then the general concept, I think, was one that they advocated.

GRW: And that came out of an NIH/NIGMS program that was nationwide, as I recall.

JAO: Yes, it was based on this proposal in *Science*. There was a Congressional line item for Centers in Pharmacology and Drug Toxicology so that NIGMS had that slotted into their budget by Congress, and it was a time when they very much listened to Shannon, not directly, but off the record.

JDM: And how would you say that the funding of that Center changed the Division?

JAO: The key thing that it did was allow us to fill our Division with respect to faculty and equipment resources. So, it supported people like Allan and David, once he came out of the fellowship and moved on to the faculty. It allowed us to recruit Grant. All the time we were triangulating and my salary was on there in the beginning and as I got other grants we moved it off and used that money, but some of it stayed on the Center. Somehow the now senior people, but initially as junior faculty, were funded by the grant, and as they achieved seniority, they were able and expected to fund themselves to a greater extent so that this could free up money for junior people to emerge with the Center funding. So, faculty development was important and we gave some support to Jim Dingell, who helped us a lot in the drug metabolism area and, of course, Grant's program when he came. We did not have a mass spectrometer in the initial application, but, as I learned over the ensuing months of the value of mass spectrometry in drug metabolism and through contacts in Sweden here it had been employed so successfully, we thought we ought to include a mass spectrometer, both for studies in drug metabolism and for work related to our general biochemical pharmacology goals and objectives. I guess prostaglandins at that time were only a glimmer so the main thrust was drugs. But we had already the concept of using the mass spectrometer with stable isotope methods and I had become infected with the idea by Udenfriend; he liked to come into my lab and

just chat about things and he told me about his mentor in biochemistry, when he was a graduate student, who had developed one of the first stable isotope dilution methods. Then, Udenfriend had helped Ralph Peterson, who was an endocrine fellow at the NIH, to develop the first stable isotope dilution methods for measuring steroids. He and I discussed those things extensively. Those were all radioisotope based but when I became acquainted with the mass spectrometer, I saw the potential for using deuterium-based mass spectrometric methods for isotope dilution and that as one of my objectives in getting the mass spectrometer as part of the Center. So, we had originally requested other equipment, I think, including an electron microscope but as my priorities changed, we shifted to the mass spec. Allan Bass and the Pharmacology Department's support were absolutely crucial for that development because Allan allowed us to recruit a tenure-track faculty person to be in charge of the mass spectrometer lab.

JDM: And what year was that?

JAO: I don't remember the exact year, but it was before I went to Stockholm and a year or two into the Center grant.

GRW: Jack Watson came about '69 or '70.

JAO: Yes. I went to chemistry and asked them if they would like a major responsibility for developing high resolution mass spectrometry while we, in the medical school, developed standard mass spectrometry. Either for lack of interest or resources or both, they said "Why don't you take the lead and we will support it with a joint appointment for the individual who heads up the mass spectrometry lab." And, of course, this was right

across the parking lot from the chemistry building so it was a convenient location. So, when we recruited Jack Watson, who had been with Beaman at the MIT and had done an organic chemistry post-doc in France, the program got off and rolling. Then we began to do stable isotope dilution methods for the prostaglandins by the time the instrument got set up and rolling. The primary prostaglandins and by that time I realized we'd have to go for the metabolites and that led me to take off to Stockholm in 1973 and to learn how to undertake the biosynthesis of those stable isotopes.

JDM: So it seems like in the late '60's early '70's, the interest was clearly evolving into several areas. One, drug metabolism, which continued with the recruitment of Grant and other people, and then the eicosanoids, which was an area that you seem to get interested in. How did that evolve?

JAO: Well, I was interested in vasoactive substances. I guess, hypertension had been my clinical interest involving catecholamines, serotonin and histamines. When the prostaglandins emerged it seemed that these might have cardiovascular importance. They were so exquisitely potent and having spent time with bradykinin, I should mention bradykinin as another vasoactive substance, and my first grant at Vanderbilt was on bradykinin and kallikrein pharmacology. But having exercised myself with smooth muscle strips as a method of analysis, that also being the approach to the prostaglandins at that time, I was really motivated, but if I was going to get into that field, I didn't want to do it with bioassays. Of course, that's what made John Vane (laughs), but my objectives were to be able to study the prostaglandins in humans and I knew I wasn't going to make headway with trying to measure in blood with bioassays, or even with the radio-immuno assays that were coming on stream, so that led me to invest in the high

chemical methods for analysis.

JDM So that prompted your, in the early '70's, going to Bengt Samuelsson's group. Tell us a little bit about that experience.

JAO: Well, as I mentioned, we had this stable isotope methods for the primary prostaglandins in development or developed here and I recognized that we needed to get the metabolites. Bengt had just elucidated the metabolic pathway of PGE so I went to Stockholm and visited with him at that time. He was head of Biochemistry at the Veterinary College in Stockholm. If you start out at the Karolinska, you can't become Chairman by moving up in the ranks, you have to come in from somewhere else, so he did a lateral arabesque and I met with him there and was interested in learning about what he knew about the metabolism with the idea of developing a stable isotope. But by the time I got there, it was clear he already had developed the PGE stable isotope.

to be continued; part II taped on January 21, 2005

Professor John Oates' Oral History of the Division of Clinical Pharmacology recorded in the winter of 2005 (January 21, 2005) at Vanderbilt University and the questioners in this history taking are Professor Grant Wilkinson of the Division of Clinical Pharmacology and Professor Jason Morrow Chief of the Division of Clinical Pharmacology

JAO: Before I went to Stockholm however, Jack Watson came to join us and we set up the mass spectrometry laboratory, and we plunged into the analysis of PGE₂ itself. We recognized the problems of measuring prostaglandins in blood. We initially targeted PGE₂ in the urine as a reflection of renal prostaglandin production and we did that. We used a stable isotope derivative of PGE₂ and were able to develop a method for analyzing PGE₂ in human urine which we published in the JCI just before I went to Stockholm.

Following up on that, with Alan Nies as a participant, we showed in the dog with renal stop flow experiments that PGE₂ came from the kidney and not from glomerular filtration. That's the data that defines E₂ in the urine as being primarily derived from within the kidney. That was the status of things before I went to Stockholm. The reason I went to Stockholm was to learn more about how to develop the methods for measuring metabolites because we recognized that PGE₂ in the urine was interesting from the standpoint of the kidney but it didn't tell us much about total body biosynthesis. Because of the problems of analyzing eicosanoids in the blood with artifactual production and extremely high clearance, I thought we'd put all our effort to measuring metabolites. So on my initial trip to Stockholm I'd found that Bengt Samuelsson had already elucidated the metabolic route of PGE₂ and following that I made arrangements to go back there as a visiting scientist and worked in Bengt's laboratory with Krista Green. We did a project that in retrospect was fairly trivial. We elucidated the presence of a tetranor-metabolite of PGE₂ in the urine. I thought because the kidney had, in particular, beta-oxidation as a metabolic pathway, that a metabolite derived only from beta-oxidation might be a stronger reflection of renal biosynthesis. So that was a project that was a fairly simple, straightforward thing that allowed me to learn how to go through all the steps that they used in the biosynthesis of metabolites and making stable isotope labels for them. So the project was published. It wasn't of any great importance but it familiarized me with all the methods they were so particularly good at and gave me hands on experience with mass spectrometry, the same kind of mass spectrometry that we had here. So that was good. It is hard for a Division Director to get hands on experience with much of anything staying at home. In essence, this allowed me to be a postdoctoral fellow. My boss in the lab there was the technician in Krista Green's laboratory; she taught me what to do everyday. That was a good experience and a chance to get my hands wet, so to speak, in

an outstanding laboratory. So when I came back we took two major directions, initially. One was we set up the analysis of PGE metabolite that already had been developed by Matts Hamburg and Bengt. Matts, I should say, Bengt's associate in Stockholm, was *extremely* helpful. He was one of those scientists that would give you all the help you would possibly need in solving a problem. He took me through all of his notebooks, all the problems in measuring PGE metabolite. I had a good handle on that even though I didn't work on it in Stockholm. We set that up here, made a few minor modifications to it, and although it was a very time consuming, cumbersome method, it worked extremely well. It allowed Hans Seyberth, working with me, to do several things. One was the demonstration of elevated levels of PGEM in patients with lung cancer and hypercalcaemia. At the time our interest was initially focused in the hypercalcaemic aspect of things. Patients in whom we found markedly elevated PGE metabolite levels were predominantly patients with lung cancer. I think that probably is the first description of the overproduction of PGE in humans associated with lung cancer. That was work which we collaborated with John Potts at Massachusetts General Hospital; to evaluate these patients with lung cancer and show that it was not a parathyroid related problem.

Another collaboration we set up was with John Gill and Fred Bartter at the NIH on Bartter's Syndrome. We analyzed the PGE in the urine and PGE metabolite in patients with Bartter's Syndrome. We provided evidence that there was an overproduction of PGE in these patients who had extremely high renin production. That was important in leading us into the whole area of the link between PGE and renin and the subsequent demonstration that renin biosynthesis was driven by prostaglandins. The ultimate upshot of all that was when Hans Seyberth went back to Germany, he discovered this in antenatal Bartter's Syndrome which is treated with non steroidal anti-inflammatory drugs

to block prostaglandin synthesis and shuts off potassium loss and hyper-reninaemia. So that was an observation that ultimately turned into therapy. Hans was one of many good fellows that worked with us at the time.

The other line of work was the development of the investigation of the analysis of thromboxane in humans. Jack Roberts was a fellow who joined the laboratory at that time to work with us on that together with Brian Sweetman and Jack Watson. We elucidated in those studies the two major metabolites of thromboxane; the dinor which was the initial one we focused on because it was easiest to synthesize a stable isotope from, simply by metabolizing off 2 carbons by beta-oxidation, and subsequently the 11-dehydro metabolite which we discovered turned out to be the best metabolite for analysis for thromboxane biosynthesis. That not only elucidated for the first time the 11-dehydro metabolite, which has been widely used as the indicator of thromboxane biosynthesis, but more recently, is an indicator of aspirin resistance, but it also allowed us now to begin to carry out related clinical studies.

Beginning in about 1975 we also began our interest in prostacyclin and shortly after that, fellows Pierre Falardeau, Alan Brash and I developed a method for measuring the dinor-6-keto PGF₁ alpha metabolite using stable isotope methods in urine. So we now had the ability to measure both prostacyclin and thromboxane biosynthesis. At that point Garrett FitzGerald came into the laboratory and conducted the study looking at the dose response curve of aspirin. He showed quite clearly that low doses of aspirin could block platelet thromboxane biosynthesis and could reduce total body thromboxane biosynthesis, and do so at doses less than those that produce comparable inhibition of prostacyclin biosynthesis. That was important evidence separating the ability of aspirin to block

platelet thromboxane from prostacyclin. It also showed there was only relative selectivity because prostacyclin biosynthesis also begins to drop at doses of aspirin over 40mg. That work, I think, coupled with Carlo Patrono's and the group at Cornell, laid the cornerstone for the concept of low dose aspirin as an anti-platelet agent.

While we are on the topic of drugs, another study was on indomethacin which we did before we were able to measure thromboxane. We measured PGE₂ production in the platelet and we were able to very nicely relate the concentration of indomethacin in patients to the degree of total body and platelet inhibition of prostaglandin biosynthesis. As part of that, we actually worked out the dissociation constant for indomethacin and showed that there was quite a good deal of inter-individual variation in relating the concentration of indomethacin to the dose ratio minus -1 in those patients and quite different slopes of the curve for those relationships. Our attention was turned away from indomethacin after that and we didn't pursue it further.

JDM John, you have mentioned most of the eicosanoids but you fell into work with PGD₂ through PGE₂? Is that correct?

JAO (All laugh) Not really. We became interested in PGD when Oswald Oelz was here because it had anti-platelet properties. We were able to show that there was a small amount of PGD formed in the platelet. That occurred even when we treated the platelets with stannous chloride to prevent any formation from PGH₂. We were interested in that and presented a poster on it. As a result of discussions at the meeting, including one with Eric Anggaard, we came away with the idea that, yes, there was a little PGD made in the platelet *ex vivo*, but it didn't answer the question of whether there was any PGD produced

in vivo by humans. Because of the constraints on *ex vivo* experiments we really needed to be able to measure PGD. That led us to undertake the study of metabolic fate of PGD in the monkey. That was an interesting experiment because it was the first data that demonstrated that PGD had a sedative effect. The monkeys went to sleep when we infused PGD into them. Alan Nies had metabolic cages for the monkeys in which they could sit up and we could infuse them with various things. The monkeys, usually very attentive during all these things, began to snore away when we infused PGD in them. Importantly we found radiolabel in the urine. Kathy Ellis, Brian Sweetman and I elucidated the metabolic fate in PGD and knew all of the myriad structures of the PGD metabolites and about this time we began to ask the question “What was the primary mediator of the flushing and hypotension in people with mastocytosis”. We had all the data, knew the structures and knew all their mass spectra, which was key. It all goes back to patients, or begins with patients, in the end. We studied a patient with systemic mastocytosis and I think a really classical pharmacologic experiment evolved there in that it was well known and surprising that antihistamines would not stop the hypotensive episodes in systemic mastocytosis; that is, H1 blockers. Jack Roberts and I had just finished a study on Carcinoid Syndrome which we published in the *New England Journal of Medicine*. This article focused on a patient with gastric carcinoid whose tumor was secreting histamine and the flushing we thought was due to histamine. We gave the patient an H1 blocker and nothing happened. I remember one Sunday afternoon it suddenly hit me that a new class of H2 blockers may block the vasodilating effects of histamine with H1 Blockers in some sort of synergistic effect. So I called up Jack on the weekend and we came back in and set up a study which showed the H1 blockers did not block flushing alone and H2 blockers did not, but the combination did completely block histamine flushing in carcinoid patients. This knowledge provided us firsthand evidence

that it takes both H1 and H2 blockers to prevent the vasodilatation from histamine in humans. When a patient with severe systemic mastocytosis came in for evaluation and we found we were not able to block the severe episodes of shock with H1 blockers, we said “Well, we’ve got the answer to this. We’re going to give him H1 and H2 blockers together and for the first time cure this problem”. Despite dose ranging these drugs together up, we did not prevent the hypotension and the patient ultimately died in shock that we were not able to prevent. We did have lots of the patient’s urine and that led us to the classical pharmacological hypothesis that if you blocked one known mediator and you don’t have any effect on the pharmacologic response there must be another mediator there. So that’s what led us to look in the urine, and, as you implied, Jason, we were initially looking for PGE. Then in the course of doing the selected ion monitoring we discovered a new peak that was a PGD metabolite which we already had structurally elucidated and knew what it was. So that was an unusual situation where we actually discovered that metabolite by mass spectrometry. After that we found about 15 metabolites in this patient’s urine. We found at least in some of those patients who could tolerate it, aspirin or non-steroidals would prevent their flushing. Jack just told me that the second patient we studied who was having flushing in the emergency room several times a month recently had told him that he was free of flushing as long as he continued to take his aspirin. But most patients can’t tolerate aspirin or non-steroidals over a long period of time. So that was the discovery that PGD was a metabolite of the human mast cell. At the same time, it was really a coincidence that Frank Austin and I had agreed to collaborate on looking at the eicosanoid biosynthesis in the rat mast cell. Using radioactive arachidonate almost at the same time that we found the PGD metabolite in the urine of this patient with the mastocytosis, we discovered PGD as the major product of the rat mast cell and, subsequently, the purified human lung mast cell. That came to a

more interesting conclusion when John Murray was a fellow and we collaborated with the Capron group in Lisle, France. They were able to do allergen installations into asthmatic airways and with control and a contra lateral airway, and were able to show enormous increases in PGD release into the human asthmatic lung following specific allergen challenge. This not only identified PGD as a mast cell mediator *in vitro* but also in the human asthmatic airway. I guess the story is still incomplete in that the PGD antagonists are still under evaluation. Although we have a DP1 antagonist we don't yet have any clinical DP2 antagonist study to fully find out what PGD is doing in asthma and allergic rhinitis. That's a synopsis of the PGD story.

JDM With the eicosanoid portion of the Division, you also investigated the leukotriene pathway and that led to the free-radical mediated lipid oxidation pathway in the 70's, 80's and 90's, is that correct?

JAO Well, we were in the race for discovering leukotrienes. Unfortunately we were working on the wrong model because the rat peritoneal mast cell doesn't make appreciable amounts of leukotrienes. We were looking at the wrong cells. Early in my career at the NIH, I had worked with the mouse mast cell leukemia type of model in which you can inject mast cells in the mouse peritoneal cavity and they'll grow and produce mast cell mediators. I suggested to Frank that we might look at that. He was not very fond of the rat peritoneal mast cell and thought we shouldn't divert ourselves but that was a model that Bengt found leukotrienes in. We were pretty sure it was sulfur conjugate but you couldn't get enough of it to analyze with that model. So we didn't get very far in the leukotriene area. We did elucidate 15 HETE as a part of the human eosinophil, though. I think that identified the eosinophil as a major source 15 lipoxygenase in humans. It was

based on that work that the group in San Francisco ultimately cloned the human gene for the 15-LOX. The way we got into the lipid peroxidation was Jack's attempts to measure PGD in plasma. He found that in plasma there was an enormous amount of the metabolite of PGD that had an F ring structure in plasma after storage. He went back to the Burroughs-Wellcome Fund, who had funded 5 years of his work, and he asked for extended funding. He told them he wanted to look at these F-type prostaglandins in plasma and try to find out what they meant. They said "It doesn't sound to us like a fruitful pathway of study" so they didn't give him any money (laughs) even though he had been a Burroughs-Wellcome scholar before that. Believing in letting people pursue leads I did that and he found together with you Jason that these were lipid peroxidation products that accumulated when you allowed plasma to sit around at low temperatures for too long. I guess that was based on his studies demonstrating the metabolism of PGD to the F-ring metabolite, which is what he was looking for in those plasma samples. I guess you and Jack can probably tell the isoprostane story as well as I.

JDM Are there any other major areas of eicosanoid biology for the Division that you think ought to be commented on?

JAO Oh gosh, I am sure there are dozens. Certainly Alan Brash has made major contributions in the identification of novel lipoxygenases and their discovery. I think our present discovery of cytosolic phospholipase A2 mutation, a loss of function mutation, will identify a new clinical syndrome associated with gastrointestinal bleeding and perforation from the small bowel and I think will probably tell us something about the function of 'prostaglandin-less' human which hadn't been found before. Certainly our studies with cyclooxygenase have elucidated the mechanism of action of acetaminophen and more

recently salicylate and, although yet unpublished, we now know that the acetylation of cyclooxygenase by aspirin is regulated by the oxidative state of the enzyme. Aspirin can't acetylate the cyclooxygenase in its higher oxidative state which could be a mechanism of aspirin resistance.

GRW: Eicosanoid biology now permeates Vanderbilt. Would you like to comment on how that came about? Was it accidental or was it a result, perhaps, of collaboration, first with the pulmonary people and then the renal people who then went their own ways?

JAO: Early on we had a participation in Ken Brigham's Program Project or Center, I guess it was, for lung research, and out of that grew some of our collaborations in the asthma field. Larry Marnett's arrival here was based on recruitment to one of the Stahlman Chairs. I guess that was reason enough for him to want to come to Vanderbilt. I would think perhaps the fact that we had a well established mass spectrometry capability here may have played into his decision but I couldn't speak to that. He certainly was a leading figure in the eicosanoid field before he came to Vanderbilt. Ray Dubois came without any eicosanoid interest until, in his studies on the EGF dependent early gene induction process, he found the COX2 gene in colonic epithelial cells and, subsequently, without a lot of wasted time, identified COX2 in colon cancer. His rapid advance of knowledge of COX2 and colon cancer and some of his and Bob Coffey's advances were contingent on collaboration with Jason Morrow to obtain a read-out on prostaglandin biosynthesis. So once Ray made that original discovery the environment here was certainly conducive, with Jason's leadership, to develop that in a very productive way. Harry Jacobson came here with a PPG oriented towards eicosanoids and hoping to bring Camille Faulk here together with Jorge Capdevilla; unfortunately he couldn't pry Camille loose from Dallas.

However, Jorge came and continued to collaborate with him. I guess I didn't mention what we had done in the area of eicosatrienoic acids. Ernst Stohler was a fellow here working with us and Alan who identified certain dihydroxy derivatives of arachidonic acid from the kidney and postulated and published that these almost certainly came from an epoxide intermediate. They presented their results in a Winter eicosanoid meeting and the subsequent year published the structure of the epoxide metabolite in *JBC*. In the mean time, Capdevilla had also made his identifications with Camille Faulk; synthesis of these compounds which he had been doing with EJ Corey before he went to Dallas. But I think our work was really the first that identified the eicosa-epoxy-trianoid pathway of prostaglandin metabolism. Jorge came and we backed out of that field. We didn't want both of us here plowing in the same field, also I guess, not entirely generous of us, I was department chairman at the time (laughs). I had my hands full and decided to constrain my breadth of interest. That's developed into a very productive area. There's been some collaboration. The mass spectrometry facility was key in recruiting Jorge here. The opportunity certainly made it for him an attractive place to continue his work.

JDM: Well John, the eicosanoids have obviously been a major focus of the Division. I thought maybe we could shift back to some other areas and have you comment on those. For example the area that Grant and others have been involved in has been broadly defined as drug disposition and metabolism. We talked about the early years with Grant coming and others. Do you want to expand a little up to more of the present in that area first?

JAO: Picking up on what we discussed earlier on, presystemic clearance, we left this story at the point where an interest was developed in that area with Grant's coming and the team of David Shand, Alan Nies, and Bob Branch. The concept of utilizing presystemic

clearance as a way of determining the intrinsic capacity of the liver to metabolize drugs evolved into an entirely new way of examining hepatic drug metabolism in humans (laughs). Did I say that right? This allowed a metric, if you will, of human hepatic drug metabolizing capacity, developed out of a concept that presystemic clearance allowed one to relate intrinsic clearance and blood flow independently to the hepatic extraction ratio. Knowing that, it became possible to separate the effects of blood flow as a contributor to clearance as opposed to intrinsic clearance in the liver. That provided a conceptual framework for which one could examine the pharmacokinetics of drugs in humans in an entirely new light and dissect out the causes of changes in hepatic elimination. From my own standpoint it was very gratifying never to have to look at another one of these mammillary (laughter) diagrams of pharmacokinetics with rate constants going in all directions. That put an end to that era and was a great accomplishment, Grant. So that, in my mind, at least, this model provided a conceptual framework for addressing important questions in drug disposition, including the effect of aging, blood flow, liver disease like cirrhosis, of P450 polymorphisms and now transporters, and all the other things Grant can tell us about. The establishment of that approach, which allowed one to clearly identify the hepatic drug metabolism, put Vanderbilt in the forefront of quantifying the fate of drugs humans by metabolic pathway,

JDM: Now the Division, so we have it on the record, with the original center grant in 1980 or so, split off into two large multi-investigative grants. Would you care to comment on why and how that happened?

JAO: Around 1980 the NIGMS council, much to my pique, got together and decided that they wanted to put caps on center grants. As you know, at the NIH, there's always the cycling

of interest in multi-disciplinary research versus individual R01 grants. The council at that time had taken the attitude that they didn't want to put as much money into multi-disciplinary things. So they wanted to cap all center grants at about half the level we were being funded at. We were not the target of it although we had one of the large ones. And, of course, that distressed me and all of us greatly (laughs) until we came up with a plan in which the drug metabolism disposition element of it was excised and placed under Grants' direction, as well as new ideas and concepts that went into the renewal at that time. The other elements continued under the center as a largely eicosanoid-oriented center grant. At that time, we were pretty far advanced in the eicosanoid field. I think that those separate grants went in at about 1982. I remember my great concern about this in calling the NIH staff who were enthusiastic about the idea of Grant being the principal investigator for a project that included that aspect of our center because they viewed Vanderbilt as a jewel in their crown (laughs). Well that's exaggerating but they did view us as an example of their goal in clinical pharmacology and human pharmacology, in general. They recognized that there were these two dimensions and that they didn't want either one of them to disappear because it would diminish the impact of a center that they valued at the time. I think it is fair to say they did have enthusiastic support for us. That didn't necessarily win us any points from the reviewers, but at least they were supportive of us as we went through this transition and they could have *not* been. They could have said "you can only have one of these instruments". I think they saw the great quality that had been developed here with Grant and colleagues in that dimension of what had been the center grant.

GRW: It may be important to point out that the funding situation in the late 60's up to the late 70's was completely different than it is now. In the Division there was John as a senior

investigator and then there were a number of junior faculty members. The junior faculty members were completely protected from any of the pressures or stressors that are present with research. Money flowed from John we didn't know how he got it (laughter). We didn't know how he spent it but it was always there. The idea of having to get your own money was foreign. This wasn't on the radar screen until actually the cycle before the split, I think, when the NIH was again cutting back on funding. I had a project in the center which was a project on aging and when we got the approval on the funding we didn't think that there was enough to cover everything in the grant. So NIGMS allowed us to take the aging aspect out while retaining the funding for the whole center and that set me on my R01 pathway. Until that time the idea that being self-supporting, as a necessity now, was a foreign concept to junior faculty members. In its halcyon days, John was a kind father who handed out pocket money every week!

JAO: (laughs)...Only to where I thought it was going to bring an academic profit. It's probably worth interjecting at this point the genesis of the NIGMS centers and the importance of Brodie, and the other major force at this time was the Burroughs-Wellcome Fund that provided some of the funds that Grant was talking about. Initially I had Burroughs-Wellcome scholarship that freed up enough money that I was able to fund people like David Shand. David, Alan Nies and Jack Roberts were all Burroughs-Wellcome scholars. That added to our ability. When we had a bright young fellow become junior faculty members we had a means of supporting him or her, both the center and the Burroughs-Wellcome money, all without any dollars from the University during the early years. Ultimately, when Leon Cunningham was the associate dean, he recognized that our activity was of the size that should get some recognition from the central administration. He put an 'Associate Dean for Research' budget item of \$25, 000 a year into clinical

pharmacology, which was the first money we got from the University (laughs). Now, that's not to say they weren't extremely supportive. I think I talked earlier that Randy Batson took me to Upjohn and got funds for the upper floor of the Werthan Building. That really gave us the space that allowed me to stay here to build the Division to its next generation. The Burroughs-Wellcome Fund was very important and I described earlier how this came about; through Mac Harvey's initiative, who was Chair of Medicine at Johns Hopkins, had been a fellow in England with Sir Henry Dale, and who had won the Nobel Prize through his work in the autonomic physiology.

JDM: Grant if you want to add just a few remarks in this area?

GRW: No, I think one of the transitional things is that we're going to move on to the ion channels. This is interesting in that it started out in the disposition program when we were looking at anti-arrhythmic agents.

JAO: Right. Well this really started from presystemic clearance as well. The first-pass clearance of lidocaine that was being studied at San Francisco caught my attention. At that time, lidocaine was one of the more valuable anti-arrhythmic drugs in use. Every ICU had lidocaine drips flowing to try to suppress ventricular arrhythmias. I got the idea that if lidocaine was such a useful drug and it had a high presystemic clearance why couldn't we make a lidocaine analog that resisted metabolism. Fired up with that idea I went to the company that I thought might do that. It turned out my idea wasn't wholly original, that Astra were thinking the same thing.(laughs) We had a long conversation there that led to us doing the clinical trials on a drug called tocainide which was a lidocaine analog that was not as susceptible to hepatic metabolism and had a long half

life. That led us into the second phase of anti-arrhythmic drugs. I think I mentioned that my first anti-arrhythmic drug work was with a Ciba-Geigy drug, SU13197, that we found the first-pass metabolism with an extreme inter-individual variation which essentially killed the drug. So with tocainide we carried out the first clinical studies, a couple studies with pharmacokinetics and drug action. That was a study that Ray Woosley got involved with when he was a fellow and led us into collaboration with the cardiologists in the GCRC to begin to set up systems for monitoring cardiac arrhythmias in the CRC. With the support of Elliott Newman, initially, and others, subsequently, through several iterations of arrhythmic monitoring capability we were able to get 24-hour records of heart rhythms on patients. Ray Woosley was a major player in that. He was very excited, very organized and stayed behind the efforts to develop improved monitoring systems. Vanderbilt had one of the first template, as they called it, for monitoring for cardiac arrhythmias involving a computer driven analysis of an abnormal electrical signal which the computer could pick that up when it occurred and signal arrhythmia without you having to sit there and look at 24 hours of tracing. So that was a real collaboration that Newman and the CRC gave to us, something uniquely Vanderbilt that allowed that kind of thing to go on. I don't think we would have advanced without that kind of collaboration. Then it took us back to our old friend propranolol. We then did the studies with Ray Woolsey and David Shand, who was still here, which demonstrated a dose-response relationship, between the ventricular arrhythmia suppressing effects of propranolol. We were able to, for the first time, show a very nice concentration-response relationship to suppression of ventricular arrhythmias and found that the suppression by propranolol occurred at part of the concentration-response curve that was well above what was required to block the beta receptors that supported the idea that these drugs might have an anti-arrhythmic effect that was separate from their beta blocking effect. So

those studies were going on when Ray subsequently developed the capability and interest that led into a broader program. This started with the procainamide story and the possibility that procainamide could be metabolized to a metabolite that could be responsible for the development of the lupus complication. Although we didn't succeed in identifying that pathway of metabolism, we were interested in it and it led Ray to carry out the studies that showed that there was a reciprocal relationship between development of ANA antibodies and the acetylation of procainamide. It was with that sort of background that Ray, asking in a sense a metabolic question, began to look for procainamide-like molecules that would not produce lupus. He began discussions that led to our being involved in studies with encainide. We didn't do the very first encainide studies but out of our early research came the classical study in which one individual had extremely high levels of encainide, a long half life, and failed to suppress ventricular arrhythmia. That led to the discovery that it was an active metabolite which was responsible for encainide's anti-arrhythmic effect. It immediately explained why people had gotten in trouble with the intravenous administration of encainide where they would look at short intervals and it was getting no response and they jacked up the dose, each time, taking no response as an indication for increasing the dose. In fact, all the time they were accumulating active metabolites and all of a sudden, once they got an effect, they already had all the precursor drug in there that kept forming metabolites. So they got the bad pro-arrhythmic effects with the drug. If they had been to Vanderbilt before they had done those studies, they probably would have avoided that.

GRW: My memory serves me that that index patient never actually responded to any anti-arrhythmic agents so it was an example of getting the right answer for the wrong reason.

JDM: I presume that subsequently this work led to the recruitment of Dan Roden and others in the interest ion channel biology?

JAO: Dan Roden came here as a fellow from Canada. You've probably heard him tell the story that I told him we didn't have any funds and if he were only an American citizen we could put him on the training grant. He said his wife was an American citizen so that allowed us to bring Dan here as a fellow. He worked with Ray on encainide, one of his first projects. One of the things I alluded to was that everything that we were doing we were trying to look at pharmacokinetics, and in the case of quinidine, which was widely used at the time, we were interested in quinidine drug concentration in relation to effect. Dan and Ray began to look at the patients who had polymorphic ventricular arrhythmia-*torsades de pointe*. Being in arrhythmia research they were now very much involved in the arrhythmia scene clinically. They realized they were seeing a lot of patients with *torsades de pointe*. We began to study these and had the quinidine assay set up in Grant's laboratory so we could get plasma level-arrhythmia relationships. What they found was that arrhythmias occurred often at very low plasma concentrations and that it was correlated with diuretic use and with hypokalemia. I won't speak for Dan, but I think that study stimulated his desire to go to Columbia to work with Brian Hoffman to try to understand the relationship between potassium and quinidine. He had a very focused objective in that sabbatical and did the classical experiment showing that quinidine did not produce particularly abnormal polarization. When they did quinidine in the presence of low potassium, however, they got markedly distorted re-polarization after depolarization in a dog model. That opened up the whole issue that Dan has been pursuing since of the pharmacologic regulation of re-polarization in which he is a world leader. Importantly from the standpoint of bi-directional translational research, a fundamental

hypothesis arose in the area of clinical setting in which measurement of plasma concentration was part of the analysis that led to the conclusion of the importance of potassium. The other thing I will say of the arrhythmia group, which I think is tremendously important in my own personal view, is that with Dan and Ray and the rest of us who were involved to varying degrees, Alan Nies and David Shand in the early days, it was a group of critical clinical scientists looking at all this. We began, and in a particular way, Dan began to ask the question does a drug that prevents non-sustained ventricular tachycardia in reality represent an effective drug that prevents sudden death. We were obviously seeing some people with sudden death occurring during treatment with these sodium channel blocking drugs. I think it was the critical view that the Vanderbilt group took along with maybe one or two other people around the country that helped to move the question of; “is this hypothesis of suppressing ventricular arrhythmias the correct hypothesis for preventing sudden death”. The CAST trial showed that wasn’t the case. While some in the press, as we see going on with Vioxx, said, “Oh wasn’t this horrible! Giving anti-arrhythmic drugs and poisoning people! Wasn’t the CAST trial a terrible thing for the NIH to do” I think it was one of the most important trials for NHLBI in that era because it totally changed the science of thinking about anti-arrhythmic drugs. There were huge investments in sodium channel blocking pharmacology going on at the time. Major pharmacologists and electro-physiologists in the field were all invested in what happens when you block the sodium channel and this trial had turned the whole thing on end and totally reoriented the basic science that was fundamental to thinking about anti-arrhythmic drugs. While Vanderbilt wasn’t the only player, I think the fact that we had a very critical approach to thinking about anti-arrhythmic drugs. Our investigators, Ray and Dan in particular, were of the mind-set that they were prepared to question the then existing conventional wisdom that suppression of VPC’s prevented

sudden death. I think without that kind of critical thinking the NIH can't do the right thing.

JDM: There's one other broadly defined area within the Division maybe you could comment on, the area that I have chosen to define as Cardiovascular Pharmacology involving people like David Robertson and colleagues, Alastair Wood and Nancy Brown. Would you care to comment on that?

JAO: We talked about the early cardiovascular studies earlier, I suppose, in the early '70's, my own interest moved out of that area. David Robertson came here as a fellow while we were still interested in the anti-hypertensive pharmacology area. We did some studies in which he used mass spectrometry to analyze the catecholamine metabolites. We thought that might give us a more accurate analysis of the methylated metabolites of the catecholamines. He did a nice little study (laughter) I shouldn't call it a little study, it was a big study, in which he showed that patients with borderline hypertension had elevated production of metanephrines in the urine. That was something of a good deal of interest at that point in time, namely that there might be an adrenergic abnormality in early hypertension. He continued that interest to the area of caffeine's effect on catecholamine and renin pharmacology, and showed first that caffeine was a stimulus for catecholamine release and renin release, and secondly, that patients became tolerant if they took caffeine on a regular basis, that is, it no longer produced release of those pressor agents. That established David Robertson in the area of caffeine pharmacology which he and Italo Biaggioni have extended with great success since then, to the general area of purinergic receptors. The other area of cardiovascular interest that we had on Bartter's Syndrome, was, in fact, that it gave us a clue that prostaglandins were involved in renin release. We

went on to do a study with Chris Frolich and Jim Wilson in which we dissected out the role of prostaglandins in human renin release. That was a study in which we had shown that indomethacin would lower renin but we also knew it would cause sodium retention. As clinical investigators, we realized that we shut down renin just by retaining sodium. That didn't prove the point so we set up studies on the CRC in which we put people on a 10 milli-equivalent sodium diet so they couldn't retain sodium. Then we isolated the beta adrenergic control of renin secretion by giving a beta blocker. We controlled the sodium, we controlled the beta adrenergic system and in that setting we showed that indomethacin was a powerful inhibitor of renin secretion in humans. This was the most definitive evidence in humans, that in addition to the adrenergic regulation, there was the prostaglandin control. From that a number of studies emerged. Alan Nies and Bob Branch had a very productive line of research which identified prostaglandins playing a role in the macular densa regulation of renin release as opposed to catecholamines being a mechanism of signaling in the baro-receptor to the kidney, prostaglandin signaling renin release, that you got when you decreased renal blood flow and signal through the macular densa. They conducted a number of very elegant studies in dogs that Alan continued, even after he went to Denver that really nailed the prostaglandins in renin release. We did some studies where we showed that, in the dog at least, PGE was not a very good stimulus for renin release. That was known from studies with Eric Anggaard when we showed that prostacyclin was a stimulus for renin release. In tissue slices of the renal cortex, we demonstrated prostacyclin release in renin through a cyclic AMP dependent mechanism. As it turns out I think that is very species dependent. Different species depend on EP receptors and others on IP receptors. Probably in the human, the EP may dominate. That was part of our major interest in hypertension. Clinical Pharmacology's part was important in Vanderbilt's Hypertension Center, one of the early

such centers in the country that was very focused on renal vascular hypertension. His work with renin fit right in with that. Before he left here, David Shand was the director of the Hypertension Center, which had a number of cycles of NIH funding, and included Tad Inagami. That was a typical Vanderbilt collaborative effort with Endocrinology and Grant Liddle playing a role together with us in vascular research. Nancy Brown's involvement in the hypertension area came through her initial fellowship with Bob Branch who was continuing his interest in eicosanoids, adrenergic agents and renal pharmacology. It has taken off in entirely new directions of bradykinin receptor antagonists and their link to the coagulation system among other things. I will leave it to her to describe all of that but those are some of the links with hypertension that went on before Nancy and David took off. Now David Robertson was a fellow here and went to Hopkins as the Chief Medical Resident, they called Assistant Chief of Service I think. They very badly wanted him to stay at Hopkins but we were fortunate to get him back here. Victor McKusick, who was Chair of Medicine at Johns Hopkins at the time, advised David that to be successful in medicine you needed to pick a disease and focus on it. So David picked autonomic insufficiency and focused on it in an extremely successful way. He's had a number of big discoveries including the dopamine beta-hydroxylase deficiency and a transporter genetic deficiency.

JDM: It's been 40 years since the Division was founded. In terms of its success has it exceeded your expectations? Would you give an overview of your thoughts over 40 years?

JAO: The last time we talked Grant had asked me if I envisioned this becoming largest most successful program in the world and was that my goal in the beginning. I modestly deflected saying that I had not had the competitive urge to be number one in the world. In

rethinking that, I and Allan Bass also had the view that we wanted to create an academic program that was ideal for training in this field. It very quickly became apparent to me that the ideal training environment was also the ideal research environment for carrying out human pharmacology research. It was that kind of thinking, that we really wanted a complete environment for training and not just a one-person research unit where everything depended on the interest and capabilities of one person but to give trainees a broad spectrum of interest like I had the good fortune to have at the NIH. We really needed a group of people and Grant's recruitment certainly was a major component of that strategy as was Jack Watson's, to build a scientific community of scholars, if you will, that could bring different intellectual and scientific strengths to bear on the kind of things that trainees needed to know and at the same time the kind of resources that were needed to solve scientific problems. I suppose while I hadn't had the "Number One" hanging up there as a guide, I did and Allan did as well have the idea of creating an environment for science that supported human pharmacology and that led us to achieve that, to recruit and retain the kind of faculty made that possible. That got us to our present position and was fortunate.

JDM: Grant do you have any other questions?

GRW: I would like John to define Clinical Pharmacology.

JDM: Yes I would like to hear it. John, would you give us your definition?

JAO: I am always amused by that question (laughter). Leon Goldberg, you didn't know Jason, but Grant probably recalled him. He was a clinical pharmacologist who was head of

Clinical Pharmacology in Chicago who discovered dopamine's use as a pressor agent, Leon said that "Clinical Pharmacology is what I do". I suppose I could define it in the same way. One could come up with a definition that has to do with the investigation of pharmacologic agents in humans and the science that surrounds that and a commitment to that. A lot of people look at drugs from time to time in their careers when they are involved in a lot of other fields. But one of the key things about clinical pharmacology is that it's had a focus on drug research in humans. It is a sustained focus that is not necessarily subspecialty oriented. By virtue of that it can examine principles of pharmacology independent of the organ-based kind of thinking. I was very interested in human pharmacology. They put a lot of effort with John Burns, who was one of Brodie's trained people and ultimately who went on to divert at the Roche Institute, into doing things like drawing up guidelines for studying drug metabolism in drug development and from the industrial approach to trying to quantify pharmacokinetics and metabolic pathways prior to human pharmacology. They also took an interest in human pharmacology and in trying to raise the standards and interest in this across the country. So they put on a workshop initially on drug metabolism which I participated in and attended. It was about drug metabolism in a broad sense. After that they wanted to put on a workshop in clinical pharmacology and as I had been at this earlier one and I had seen how much work went into it, I got "volunteered". It was a huge undertaking with faculty coming in from all over, well funded, and having to set up experimental things for people to do here in the workshop, laboratory kind of an experience. I was really on the verge of not wanting to do this because we had research interests and we didn't want to be distracted with all this. But Allan Bass quietly persuaded me that if we wanted to assume a position of national prominence that we better undertake something like this. I realized he was right. We invested in that and put on this workshop and brought in people like

(Sir) Colin Dollery, Jim Gillette and other key players both in the basic and clinical pharmacology sciences, and at laboratory workshops. It was a good experience for our faculty. It drew people, like Jim Dingll, who was a member of the pharmacology faculty working in drug metabolism closer to us and importantly Alan Nies was one of the attendees so he got introduced to Vanderbilt, as did a number of people in his generation who came to participate in this workshop.

GRW: What year was that?

JAO: You came in '71.

GRW: I was involved in a similar one in '68 in San Francisco. It must have been "65-'66.

JAO: It was before San Francisco. It did have, as Allan predicted, the effect of giving us some visibility nationally and internationally. It was a good thing for us to have engaged in but I think more importantly it helped us recruit Alan Nies who was a star in our faculty while he was here. Then later it focused in '68 on pharmacokinetics which helped to get David Shand and through David, me interested in making a bigger investment in pharmacokinetics and drug metabolism

JDM: Thank you, John. I think we'll stop. Note this was recorded on January 21, 2005 at Vanderbilt University.

Professor Grant Wilkinson's Oral History of the Division of Clinical Pharmacology recorded in the Summer of 2005 (July 27, 2005) at Vanderbilt University. The questioners in this history taking are Professor John Oates of the Division of Clinical Pharmacology and Professor Jason Morrow of the Division of Clinical Pharmacology.

GRW: Grant R. Wilkinson

JAO: John A. Oates

JDM: Jason D. Morrow

JDM: We are here to interview Professor Grant Wilkinson regarding his recollections on the development of drug disposition and metabolism within the Division of Clinical Pharmacology at Vanderbilt. So I think to start off with, Grant, we thought we would begin at the point where you came to Vanderbilt in 1971 as a young recruit from the University of Kentucky. Why don't you just launch into what your views were on arriving, and let's take it from there?

GRW: John recruited me here in July of 1971, and I guess, in retrospect, I was the final piece of his initial recruiting scheme because at that time David Shand had moved from being a fellow to a junior faculty member; Alan Nies had come from UCSF; and Jack Throck-Watson was in the mass spectrometry area. So there was a critical mass for a drug metabolism group. John also was beginning his prostaglandin interest at the time...I can still remember him drawing those wiggly double bonds all over the place. He and Chris Frölich--a post-doctoral fellow--worked in that area, and John continued his hypertension interest, along with Andy Michelakis. My recruitment was, I think, to bring a pharmacokinetic and bioanalytical expertise to the group. David was a pretty broad human clinical pharmacologist, Alan was a more physiologically applied a person, and I brought in the quantitative aspects of kinetics. At that time drug metabolism was in its early days. It was a little ahead of clinical pharmacology, but we were still dealing primarily with drug metabolism in animals. There were biochemists like Ron Estabrook

and Judd Coon who were looking at the mechanistic aspect of drug metabolism. In addition, pharmacologists such as B. B. (Steve) Brodie, Jim Gillette, and Gil Mannering were a little bit more inclined towards the drug aspect rather than the mechanistic area, but it was a fairly rudimentary situation. For example, cytochrome P450 was thought to be a single enzyme at that time, but we knew there were also Phase II enzymes. So John, I think, was interested in moving the area into the clinical arena connected to what was a strong sense of the concept of plasma drug levels and response. As he has previously described, the latter was built on the early work in the Second World War period in the malaria program, and it related plasma levels to effects, both desired and untoward; also there was some use of pharmacokinetics to describe the temporal aspects of that situation. So in 1971, there was a group of very young, ambitious scientists trying to develop their careers and having fun in this emerging area of drug metabolism. About a year after I came, Bob Branch arrived as a fellow and he really cemented the linkages between Alan, David, and myself, because he was the “hands” that did a lot of the early work on the clearance model which we worked on. As I stated earlier, John got me here in July 1971 and if my memory serves me right, that coincided with the first renewal of the Center grant in the following August or September. I can remember we had a site visit, which was my first one, and I think we spent an afternoon out at Central State Hospital because there were some joint programs with John Davis and the psychopharmacology people out there. Also, Jim Dingell was out there doing his drug metabolism research. My traumatic memory is that I had a stressful one-on-one conversation for about 30 minutes with Jim Gillette who was trying to find out what I was going to do. Of course, at that time, I had no idea. But anyway, the funding came through, and we got on with the science. So there were a lot of Center activities, and we’ll get back to those a little later. However, John also was very interested in establishing a routine therapeutic drug monitoring

program, as it became later known. At that time, the concept was out there but, in fact, nobody had actually applied it in a hospital setting on a routine basis. So John put together a grant application to the John Hartford Foundation which was funded, and allowed us to set up a therapeutic drug monitoring lab within Clinical Pharmacology. I think John has commented earlier that the Pathology Department and Clinical Chemistry had no interest in that particular activity at that time, so for several years we were able to run this out of Clinical Pharmacology. This provided a daily service on plasma drug levels such as the anticonvulsants, digitalis, procainamide, and got the fellows involved in a consulting role. For many years it was quite an active part of the Division, until Clinical Chemistry realized that you could make money out of this and started to lean on us. Eventually we got taken over in the late 1990s, but it was a very useful means by which our fellows came to grips with classic clinical pharmacology--drugs, response levels, etc. We got a little bit of research out of it but not as much as I think either John or I anticipated at the time.

JDM: Let me ask you, Grant, if you would briefly tell us in terms of coming to Vanderbilt how it was that you came and just a little bit about your background so that we have it for the record.

GRW: Well, my wife says I tend to go into things backwards and that certainly happened here, because I was trained as a pharmacist. I did some work on the renal excretion of drugs in humans as a graduate student and then couldn't get a postdoctoral fellowship in pharmacy for a variety of reasons but managed to get to UCSF in the Department of Pharmacology with a guy called Eddie Leong Way, who John knows. Although I didn't get much out of that scientifically, I did meet Dan Buxbaum there, and we shared a

surgical table for many hours, as he taught me how to work on dogs. He was then recruited to this place in Nashville called Vanderbilt University by Fridolin Sulser, so that was the first time that I began to hear about Vanderbilt. After finishing my postdoc, I got back into pharmacy at the University of Kentucky which was a good start to my academic career, but it led me to realize that although I enjoyed teaching, it was not my real love; that was really research...answering questions and satisfying my curiosity. So after a couple of years there I started looking for jobs, and I knew about clinical pharmacology. I looked at Emory with Leon Goldberg and at Kansas with Dan Azarnoff but decided they weren't for me, and then John called and said why don't you have a look at Vanderbilt. It was the right move for me for many reasons including it was getting me back towards the West Coast; but, I haven't made much further progress in the last 35 years!

JAO: Did you participate in the course in drug metabolism that they held in San Francisco?

GRW: Yes, I was in San Francisco from 1966 until 1968, and just before I left the National Research Council sponsored...John had mentioned some other courses that they organized...a training workshop at UCSF which was drug metabolism oriented. I participated in that as a lecturer and instructor. Actually, the proceedings came out as a book subsequently, *Fundamentals of Drug Metabolism*; Alastair, I think, is the only one who still has a copy of that. I think that's when David Shand first met me, although I don't remember the occasion. Perhaps that meeting had an impact on my recruitment .

JDM: And then when you came here, Grant, did you know what you were going to do or how did you get into doing the work that you were going to do that became so well known and important in terms of drug metabolism?

GRW: I had no idea what an academic scientific career was at that time. These were very naïve halcyon days, for example the importance of NIH grants was completely unknown to either David, Alan or myself. John completely protected us, initially, from this aspect of life and was our “sugar-daddy”. John made no claims on my time whatsoever with the exception of the therapeutic drug monitoring program. Also, early on he wanted me to help out on the pharmacokinetics of guanethedine, and I did some computer modeling of that. But, in general, I had no real plan. Of course, I carried with me some past projects that I wanted to work on, but it was really the intense interaction of David, Alan, and myself that really got us going. As I said earlier, we were three young investigators striving to find niches in the academic world. Importantly, we complemented each other very well. David was an ideas man, Alan was an experimentalist, and I was the pharmacokineticist that had to bring David down to reality sometimes on some of his ideas, It worked very nicely. At that time David, paraphrasing Einstein, thought that God did not do calculus and, therefore, all of the kinetic equations that I would throw at him with differential equations and all that sort of stuff, were nonsense. If God didn’t do that, it had to be simpler, and we scratched around for quite a long time to find an alternative approach. I started to get into what is now known as physiological- based pharmacokinetics that was developed by two chemical engineers--Ken Bischoff and Bob Dedrick. This was really an advance in pharmacokinetics because it took us from an era of black boxes where something went in, something happened, and something came out-- a mysterious process where we really didn’t know what was happening--to beginning to

think of the black box in real physiologic terms. I started to play with this and at the same time over at UCSF Malcolm Rowland, who was a year ahead of me in graduate school at the University of London, was having the same sort of ideas. He came up with an initial model which he was very generous in sharing with us ahead of publication. However, because intuitively it didn't feel right, we didn't believe it. So we modified the Rowland model based on the Bischoff/Dedrick ideas and that is when we started to make the big break-through. We could now start to talk about clearance in a simple way that only involved addition, multiplication, and division--David liked that since he was able to do such mathematics. For a period of about a year we worked very hard both theoretically and experimentally with this model which, you know, is now called the intrinsic clearance model or well-stirred model for hepatic elimination. Remember that at that time there was no such thing as laptops, instead there was a computer center with a mainframe computer and a satellite printer up on the eighth floor of the Learned Building where biostatistics was located. I was very fortunate that in electrical engineering there was a graduate student who had developed an analog computer which actually was written as a digital program. The importance of that was that in my graduate studies I had worked with analog computers to describe drug kinetics, so I could understand analog computers and write the appropriate programs. I used to lurch up to the 8th floor of the Learned Building with a pile of punch cards, feed them into the reader and then wait a couple of hours for the results. The printout was just numbers, since there was no such thing as graphic programs. I used to sit down and plot everything out by pencil and paper. It was always very satisfying to me, because after a couple of runs you could now start to see patterns, and could see where the next point was going to go. We certainly realized that we were at a "eureka" moment because we could now start to predict a lot of things that previously had never been explained. But, more importantly, with Alan's and Bob's

involvement we could actually do the experiments to show that we could change certain parameters, and they would have these predictable effects, and low and behold they did. So that was the genesis of explaining the pharmacokinetic behavior of drugs on the basis of their intrinsic clearance—a paradigm shift in the field.

JDM: And what were some of those things that could never have been explained before in your mind in terms of important contributions?

GRW: Well, as I said, the big thing was putting physiological concepts into the model so that instead of drug removal from the body being a fractional rate constant, as I say to the students with the units of reciprocal time, we now got a better handle of the process, namely, clearance. Not only that but we could have the clearance determined by physiological factors such as, in the case of the liver, hepatic blood flow, binding of drug in the blood and the intrinsic capacity of the enzymes to metabolize the drug. The key thing was putting those three determinants together in the right way. Intuitively you can say obviously the factors must be important, but how do they fit together? That was the key contribution and also there was the idea that we could start to get a grip now on not only clearance but also an extraction ratio across the liver. In turn, this led back to the question of how did drugs not only get absorbed but also metabolized and removed prior to reaching the systemic circulation, which developed into the first-pass effect. So with those two ideas--first-pass effect and hepatic elimination controlled by determinants that we understood--we could now start to rationalize why John's earlier studies with SU-13197 had a high first-pass effect. That was because it had a high intrinsic clearance and large hepatic extraction ratio. Also, we could now rationalize why an oral drug had large interindividual differences in its bioavailability and yet no differences in systemic half

life because, under those circumstances, the determinant of liver blood flow was more important than the intrinsic clearance of enzymes. So it started to put together this whole package that allowed us to predict drug disposition after oral and intravenous administration based on the magnitude of the intrinsic clearance of a drug, its binding and also, to a lesser extent, the role of liver blood flow. An important part of that, I think, was that the four of us were actually very effective salesmen of our model. There were also other people working on different models which were just as valid but more complicated mathematically; however, we were able to sell this simple arithmetic model to people fairly effectively, and that's what got our thing going here.

JDM: And that, then, as you said took you up into the late '70s or even past that, Grant?

GRW: I guess until the late 1970s we continued to work on these basic concepts that are now part of the dogma. So we had a pretty good run with that intrinsic clearance model. Alan I think at that time was also getting increasingly involved with John in the prostaglandin program prior to his departure to Denver, and I started to get another line of research which was related to liver disease. In retrospect, it was very fortunate that Steve Schenker, who was chief of gastroenterology at the VA, was interested in liver disease and was wanting to get a quantitative handle on the effects of liver dysfunction on drug metabolism. My first postdoc was Uli Klotz from the Margarete Fischer-Bosch Institute in Stuttgart. He came over at the right time in that Steve and I were thinking of collaborating together, which we did for a very productive period of almost ten years. I would have fellows and technicians in my lab working out the analysis of various compounds, while Steve's fellows, who at that time had to do at least a year of clinical research, were involved in studies looking into the effect of cirrhosis or acute viral

hepatitis on drug metabolism. That particular program blossomed very well, and it was also fortuitous that Bob Branch had a background in hepatology while he was at Bristol, so that was another consolidating factor that allowed that program to move forward. It allowed us to put a little more rationality on how liver disease, particularly cirrhosis, affected drug metabolism. At the time there were lots of reports, but no underlying understanding of why some drugs were affected and some weren't, and by doing studies with drugs that had varying types of metabolism and varying intrinsic clearance values, we were able to start to sort that out. The P450-mediated metabolism was more dependent on liver function than Phase II metabolism glucuronidation, in particular.

JDM: And you mentioned that, at least early on, it was you with David Shand, Alan Nies, and then Bob Branch arrived. Who were some of the other people who contributed in the early days into the '80s? You mentioned some cytochrome P450 work with...

GRW: Well, when I came Bob Rangno, Russ McAllister had just left but Gwyn Evans was still here and was very instrumental, not only in the social life of the Division but also scientifically, working on some of the work with David and me. Anders Rane came in the late 1970s period. He came from the Karolinska Institute, with whom we still have good ties, and spent three years here mainly in drug metabolism. He did some work with indomethacin, but he was important because his work was very instrumental in allowing us to predict hepatic clearance from *in vitro* measures of enzyme activity. That's something that took a longtime to get embedded into the drug industry, but it is now a routine part of the development of drugs, that is, *in vitro: in vivo* extrapolation. Dennis McDevitt was here in the mid 1970's and subsequently he went back to Dundee and ran the unit there for many years. John McEwen was also from Dundee and went back to

help Dennis. Dennis came in, and this is interesting because it comes back to how the Division's research was all interlinked to a certain extent. There was a distinct prostaglandin program involving John, Chris Frölich, Bob Branch, and Alan Nies, but drug metabolism also had the overlap with Bob and Alan. I don't know how we got it-- John knows more about that--but there was a lidocaine derivative being developed...tocainide...by Astra. If I remember rightly, the work followed on from the SU-13197 study that John described earlier. Tocainide was pushed as a better lidocaine mainly because of its resistance to metabolism because of the substitution on the alkyl chain. This, I think, was our first formal study where we did Phase I studies in patients with arrhythmias and looked at PVC management. Dennis was largely involved in that. Subsequently, of course, that led on to Ray Woosley's research when he came into the program and continued in that area, which, in turn, led to Dan Roden and that whole segue into antiarrhythmics and ultimately into the electrophysiology and the molecular biology of ion channels. So what started in the drug metabolism area developed into a now major program. A lot of fellows were involved in that as they worked up to the CAS Study and then the CAPS Study at the national level.

JDM: You mentioned, Grant, that initially you were part of John's center grant and then I guess in the early '80's...is that when you split it off into a separate PPG?

GRW: Yes, as I said earlier, Uli Klotz was my first fellow, and the first drug that we looked at in liver disease was diazepam, or Valium. This was because the sense of the gastroenterologists, at that time, was that sedatives were bad to give to people with liver disease. So we looked at diazepam and found indeed cirrhosis did impair diazepam's metabolism quite significantly, but we came across an intriguing finding. This was

because we were looking at both acute viral hepatitis and cirrhosis and we had matched controls. Cirrhotics tended to be older than the acute viral hepatitis, so in our control population we had an age range, from about 30 up to 70 years and, much to our surprise, the half-life of diazepam increased linearly with age. At first we couldn't understand this but we did the kinetics and found that it had nothing to do with clearance as such. Aging did not effect the clearance of diazepam but its volume of distribution changed with age for some reason that I still do not understand. It was again one of those nice serendipitous observations that you take advantage of. So along with the liver disease studies, we also set up a parallel track with Steve Schenker doing aging studies. So, I guess, when the Center renewal came up in about 1977, we had a project that was going to look at the effects of age on drug disposition. The grant got funded but with a reduced budget, so John made the suggestion that we withdraw that project and put in a separate R01 for that, which we did, and that started the aging grant. At the next competitive renewal in 1982, the Center budget exceeded the recently defined ceiling amount for such awards, so John and I agreed to divide the science. He would continue to direct the Center with a focus on eicosanoids, while I would submit a new PPG to encompass the drug disposition aspects of our research.

JDM: And who were the investigators on that first PPG? Do you remember?

GRW: Well, there was myself and Bob Branch. David had left by that time and he was at The University of Florida in Gainesville. I'm not sure where Alastair fitted in because he, Bob Vestal and Dave Kornhauser had come in around the late 1970s period and were involved in some of the aging research, so he could have been an investigator.

JDM: That was the focus of that first grant, was then the aging?

GRW: No, the aging research was supported by my R01. It was different in the late 1970s and early 1980s because the PPG, although it had to be cohesive, didn't have to have such strong and focused interrelationships as it now does. So we were able to put together projects that were connected but not too closely inter-related and so there were studies on liver disease, another on anti-arrhythmic drugs and a pharmacogenetics project. I don't think you could do that today, but it was possible then. This involved Bob Branch, Ray Woosley, possibly Alastair, and myself.

JDM: And then over the years, I guess we're up into the '80s, then, Grant, where did this area of the Division head and what would have been the important contributions since that time in your mind and who were the players that have been involved?

GRW: The general theme that was starting to develop then was trying to define and elucidate determinants that effected drug disposition, particularly drug metabolism in humans. That was what we were good at and we were able to focus on it. And so we'd gone through liver disease, we'd done aging, we'd played around with kidney failure one time. Then another serendipitous event took place that got us into the pharmacogenetic area at a very early time. In the late 1970s, Adrian Küpfer came over as a postdoc in Bob's lab, and he had done studies in dogs with mephenytoin and was interested in the chiral nature of the drug and its stereoselective metabolism. He wanted to continue that work which was unusual because we didn't usually allow postdocs to tell us what to do, but, in this particular case, we thought there was something in that. In particular we wanted to extend the studies to humans because stereoselectivity in drug metabolism was becoming an

important issue at that time. Accordingly, we did studies in humans with mephenytoin in a typical study of six healthy normal male volunteers. The study was going along wonderfully when one subject complained that he couldn't keep awake while he was studying for exams and so a note was made of that. When the data came out of the lab, it was clear that his metabolism was completely different from anybody else's, and so we pounced on that and started to recognize that there were two phenotypes for mephenytoin metabolism. Now there are two things you've got to recognize. First, genetics was the old type of classical genetics where you defined a phenotype and then you tried to find out the genotype and what the mechanism of the genotype was. The second factor was that the debrisoquine or cytochrome P4502D6 polymorphism had just been described a couple of years previously, and we had started to get involved with that with encainide, another antiarrhythmic drug that came out of the program here. So we were attuned to the possibility of genetic polymorphism and wanted to pursue that and we did that in two ways. We stimulated Fred Guengerich's interest in whole issue of human genetic polymorphisms. He had previously been successful in isolating several forms of P450, and so we wanted to find out the biochemical mechanism, and he was very helpful on that and set up another track of research. And then also we wanted to go through the whole characterization that goes along with phenotyping and drug metabolism. So we set up some fairly sophisticated analytical procedures that allowed us to measure each of mephenytoin's enantiomers separately and started to find out the frequency of poor metabolizers in this polymorphism. We did that in a white population here. Pete Wedlund was very important there. But we also had a Japanese anesthesiologist, Ko Nakamura, who went back to Japan and helped us look at a Japanese population. This was our first inkling that allele frequencies varied according to racial ancestry, because the frequency of poor metabolizers (PM) was much higher in Japanese than it was in the

Caucasians, and so we played that game for quite a long time. It was frustrating that Fred never really pinned down the biochemical basis of the polymorphism—he was successful with CYP2D6 to a certain extent but less so with mephenytoin hydroxylase. It actually took ten more years after we discovered the genetic defect to the phenotype to find out what the molecular mechanism was involving CYP2C19. So that's how we started to get in to the genetics in the late '70s.

JDM: And then, since that time, Grant,...I guess since the '80s, where has this component of the Division gone and who have been some of our more recent people that have made important contributions?

GRW: Well, I think before we get there, it's important to note that we began to recognize that genetics could have a greater effect than just CYP2D6 and CYP2C19. Bob, for example, started to get into the disease susceptibility area related to drug metabolism, and for a number of years we had collaborative studies going on looking at such things as bladder cancer, scleroderma, and lupus erythematosus to see whether the frequency of the phenotype was different in these particular diseased patients compared to what it was in healthy patients. Bob carried this interest with him when he went to Pittsburgh, and we dropped it as an interest here. Then because of the observation that the PM frequency was different in different populations, we started to get an ethnic interest which was picked up particularly by Alastair and some of his fellows. Hong Hao Zhou, who eventually went back to China and is now director of an institute over there of clinical pharmacology and a senior person in Chinese pharmacology, was important in this regard. He was a very stubborn fellow who insisted in doing his own project for a while, and he had asked Alastair more than once why we gave so much propranolol to our patients. Alastair said,

that's what they need, and Zhou said that in China we give much less. Eventually he wore us down, and so we did a study in Chinese subjects comparing propranolol's disposition and also its pharmacodynamics to those in Caucasians. That was a good piece of work because it showed there were two separate ethnic/racial differences. First, the pharmacokinetics were different and secondly, propranolol's pharmacodynamics were different in the two groups. Moreover, they actually worked in opposite directions. So Alastair took off from there with his ethnic studies of drug metabolism, extending them subsequently to Phase II, particularly the opioids. I always remember, several years ago after we were into studying genetic variation, I asked Alastair, why aren't there any in the beta adrenoreceptor? He replied that he didn't know, but, unfortunately, he never did anything about it for many years, until he moved into that particular area with Mike Stein. So we were always probing for new determinants, and we were very fortunate always to find them a little ahead of the rest of the field. I think that's what kept us at the top for a period of time.

JDM: Does that looking for different determinants, explain in part the past decade with Richard Kim then and the transporter issue?

GRW: Yes. As John has said, it's always good to make discoveries but I think good scientists are extremely curious people, and they have to have answers to those questions. Somewhere in the early 1990s I came across a drug that Upjohn was developing called ditekiren, which was a hexapeptide. It was a renin inhibitor, if I remember rightly, and one of the early peptides to be semi-commercially available, and I could not understand first how a polypeptide was orally absorbed, and secondly, how it got into the bile unchanged. So we started a small project to look at those questions. Dayo Adedoyin did

some of the early biochemical studies with isolated perfused liver preparations, where we showed the elimination of the drug had all the characteristics consistent with the involvement of an active carrier transport system. Then Harumi Takahashi came in subsequently and very painfully, because this was not an area where we had any expertise, started to get into the biochemical pharmacology of making vesicles and doing classic uptake and efflux sort of studies. At about that time, Richard joined the lab and helped out there, but he was very frustrated because we could both see that we couldn't go much further, unless we committed to the then emerging molecular aspects of transporters. All that was known at that time was that there were active transport systems, and that they could be categorized into various subclasses. What they actually involved in terms of the actual proteins was not known, and to get to that stage, we'd really have to make a major commitment, and I wasn't able to do that. However, Richard was and he started to read the literature and found out that Randy Blakely, who was at Emory, had this wonderful vaccinia expression system that would allow us to clone and express specific transporters, and so he went down there and brought the technology back. I let him go in the laboratory and we started to get into the P-glycoprotein area pretty quickly and again we were fortunate. We were a little ahead of the pack, and were able to make some important contributions, first of all with the HIV protease inhibitors and then with the genetic variants of P-glycoprotein. Then Edna Choo came in and did studies that showed that we could modulate P-glycoprotein activity, particularly that in the blood:brain barrier, by giving inhibitors, which subsequently led to work that is still on going in humans now. Danny Kurnik is trying to increase brain levels of drugs by inhibiting P-glycoprotein. So Richard's program developed from a very small observation, and then was just expanded because he's good, had a lot of fellows in at that time, and it was a fruitful area that we discovered before the rest of the field. And then

the other area that we haven't really touched on is the *in vivo* probe area. By the 1990s, P450 was now known to be a family of enzymes with specific subfamilies and individual enzymes which had limited substrate specificity and so we started to try to characterize these individual forms, *in vivo*. You've got to recognize that the pharmacogenetic studies with debrisoquine and mephenytoin, that is, defining a phenotype was using an *in vivo* probe, in one case debrisoquine and the other mephenytoin, and coming up with a trait measurement that allowed you to say whether an individual was a poor or extensive metabolizer were important. Such studies showed that there was bimodality in the distribution of enzyme activity, but then I started to get interested in applying that concept of phenotyping to enzymes that were unimodal in distribution, but had a wide baseline range of activities. We started out with CYP2E1 which was stimulated by Fred Guengerich's observation that chlorzoxazone was a specific probe for CYP2E1 using human liver microsomes. We took the observation and applied it to human studies. Richard started to get involved, along with Diarmuid O'Shea, and that came out quite well; however, our efforts in the cancer area weren't very productive. At the same time we had, through Bob's work, used the hydroxylation of dapsone as a measure of CYP3A activity, but that wasn't playing out in my mind, and so we started to use the erythromycin breath test, and found that too wasn't a very good marker. Mark Kinirons did that work. Then we moved on to midazolam, first working with Kenny Thummel at the University of Washington. We started to characterize midazolam and its hydroxylation as a probe for CYP3A both orally and intravenously. Again, this came back to earlier work since there was a large first-pass effect. The question was could we quantify this first-pass effect? This led on to quite a lot of interesting findings as we started to reinforce things, including the pharmacogenetics of CYP3A to see whether certain variants had an effect on the metabolism of a compound like that. Then more

recently, of course, we worked with Harumi Takahashi who had returned to Japan and is looking at CYP2C9 and warfarin and the variants of warfarin that actually differ in Japanese and Caucasians. More recently she has discovered allelic variants in the vitamin K epoxide reductase enzyme and found that this is probably the reason why Japanese need less warfarin for anticoagulation than do Caucasians. So over the years, there has been a developing interest in individual determinants each adding to each other to get a matrix sort of thing. I guess the final thing to bring us up to date is the involvement with David Haas in the ACTG world that started about five years ago, where in order for Vanderbilt to get an AIDS clinical trial unit funded through NIH they required that clinical pharmacology be on that grant. So although neither Alastair nor I knew anything about HIV, we agreed to set up a pharmacology lab to help the national network. I convinced Alastair that instead of setting up a therapeutic drug analysis laboratory for anti-retrovirals, what we ought to be doing is collecting DNA and analyzing that for genotype/phenotype response outcomes in AIDS patients. So for about four years we've had an active role in the ACTG program that eventually came to a close because they ran out of money or more correctly they didn't have as much money as we thought we required to continue. However, the unit is still here since David Haas took it over, so our plan to have this DNA database on a national basis so that retrospectively you could go back and look at the genetic aspects of response to HIV therapy still is in place.

JDM: John, do you want to ask any questions?

JAO: I was just reflecting on how much drug metabolism resonates with the rest of the activities of the Division. Grant was talking about the work going on in first-pass effect

actually that led us to acquire the tocainide which opened up the antiarrhythmic drug area for the second time after SU-13197. With our cardiovascular background we were very interested in antiarrhythmic drugs and lidocaine was the most employed drug in the intensive care setting. Ken Melmon and the group in San Francisco had done very nice work to show that it had a very high first-pass effect and having followed that with the work here in first-pass kinetics, led us to hypothesize that we could find a lidocaine-like drug that would be resistant to first-pass metabolism. We went to Astra and asked them if they had any compounds on the shelf that were conjugates of lidocaine that we might study for first-pass metabolism. Their answer was not only that but that they had some that they were about to introduce into Phase I studies. So that's how we came into the thing that opened up our antiarrhythmic drug evaluation activity with Ray Woosley, Alan Nies, and David Shand as part of it in the beginning. With cardiologists' collaboration we, ultimately, got computerized arrhythmia monitoring as part of the activity. But it all came about because of the interest here in the first-pass metabolism. I hoped that we could develop a new drug for the arrhythmias. It turned out that the drug had some allergic adverse effects that ended its career, but, in essence, it was the first-pass interest that led us into the program in arrhythmia, and then it came full cycle with encainide, where again the serendipitous discovery of a patient who responded differently to encainide was found to have a CYP2D6 PM phenotype and very high levels of unchanged drug. It was then deduced that the drug acted through an active metabolite...that a metabolic step was required for it to be effective, so the arrhythmia area again was resonating with the drug metabolism activity to make a discovery which I think really bumped up the whole arrhythmia enterprise here because of the discovery. I say serendipitous which I think probably underplays the importance of doing studies in

humans and taking advantage of opportunities that come out of that. It is more than accidental.

GRW: I think one of the changes when I first came here was a mindset change that my species of choice was no longer the rat or the dog. It was the human, and I've never changed after that. Animal studies are important when you can't do certain things in human but, you know, over the years the ability to do drug studies of whatever form in humans subjects or patients has been our strength. I wouldn't say that was unique to us anymore but it has certainly been a major, major strength. The resources such as the CRC have been incredibly valuable in that regard.

JDM: Did you realize that early on by being here with David and Alan...that was the impetus for it?

GRW: Oh yes. I mean the concept that I as a Ph.D. could direct clinical research was certainly novel.

JDM: I want to just ask you, Grant, in the last few minutes just a little bit. In addition to your scientific contributions to the Division you had a role for a number of years in helping John run the Division. Also, I know a number of fellows that not only worked in your lab but were in the Division owe a lot to you in terms of their training. Would you care to just comment briefly on your role as Associate Director of the Division and how that evolved?

GRW: Well, you know, part of the secret of successful collaboration is probably to know your own weaknesses but also to understand others' strengths, and to combine those strengths. I don't think it is any secret that John's strength is not in dotting the "I s" and crossing the "T s" of certain issues, whereas my avocation, if you will, is attention to detail. And so when John's time became more limited within the Division because of his other responsibilities, particularly in the Department of Medicine, we made a good team in that the big issues could be developed by John but making them work, etc., was my kind of job on the floor, when he wasn't there. Out of that, I think, the Division grew well, since we both had a clear sense of what we wanted out of the Division, namely, excellence. That's the only standard that we both had and so that was very clear. Other than that, you know, I just tried to keep people from fighting. We are all prima donnas and have big egos, and so keeping the place calm and letting everybody know they're important and looking after their squeaky wheels was important. The other important thing to recognize and appreciate is that trainees are fun because they are young but inculcating them with this idea of excellence is very important to me.

JDM: Good. John, any other points?

JAO: I thought perhaps you could amplify on development of some formal didactic activity under your direction that came to be a standard for the fellows' training.

GRW: Well, I think one of the characteristics of the Division is that it has never had a very clear or well-defined definition of what clinical pharmacology is, or what its practitioners are. So over the years we've had a really diverse group of individuals and, yet, you know, I think all of us recognize there is something that could be called core clinical

pharmacology that has to be transmitted through to the fellows. That's one thing, and in the absence of a didactic curriculum we've tried to get the fellows to participate in courses which are beneficial to their further education, whether it's receptors or drug metabolism, pharmacokinetics, etc. It seems to many of us that if you get a credential of having been at Vanderbilt for two to three years then there is something that Vanderbilt has taught you. Specifically, those things that Vanderbilt is known for such as drug metabolism, prostaglandins, and you ought to come away with some element of knowledge in those areas. Accordingly, I tried to put together an informal training program that gave the fellows an opportunity to at least look at some of those areas.

JAO: I would say that has been over the years a key strength in our always high evaluations of our training grant by the NIH.

JDM: Well, I think with that, Grant, we will stop...were there any other major issues?

GRW: No, I don't think so. We covered most of the things that I had notes on that were, I thought, important.

JDM: Well, I think this is a very important addition to and we'll bundle it with John's history and, I think, have a nice thing for the Division, but also the Institution. As we discussed they are very interested in us giving it to them, so...

JAO: Speaking of institution, maybe we might discuss one other point. As I listened to your collaboration with gastroenterology, with infectious diseases and the AIDS program, with anesthesiology through Margaret and Alastair and others, I was struck by the fact that

such activities have extended and strengthened other important domains within the institution. Do you want to comment on that further?

GRW: You know, clinical pharmacology is a so called bridging discipline so you kind of expect that type of metastasizing. It has been nice over the years to see that other units have started to work in the same way, and now that's the mantra of the Institution, so it's rewarding to see that Clinical Pharmacology was, indeed, an early proponent of and player in that. Sometimes I worry that we are now becoming too narrowly focused in our research that we don't look out more widely, but I guess that's the reality of the 21st Century science. We're too compartmentalized.

JAO: Well, things do cycle.

JDM: I guess you're right, Grant, that things have become more specialized in that regard. I do think I would have to agree with you and John, though. The Division still represents an ongoing opportunity for collaborations. If we even look today at how many we have outside of this Division, I think there's very little doubt about that. Plus, I think John said, but maybe you want to comment a little bit on it about the unique interaction between medicine and pharmacology over the years in the Division.

GRW: I think it's very critical that the Division be a joint division between both departments. Because I was hired into pharmacology, I have a bias that pharmacology ought to play a larger role than medicine. Not specifically here, but in general. I mean we've always recognized that pharmacology is to a large extent where we get our science from, and one of the pleasing things was, when Clinical Pharmacology seemed to be under threat here,

it was the basic department that came to bat, and that's very important. Again, it's not as easy in these days, as it was in years past because we're spread out. We have different interests, but I think it is very critical that we keep these ties to both departments.

JDM: Yes, I think that's a very important statement that you make.

JAO: Since you mentioned pharmacology, I guess it's probably worth pointing out that something that was unique here at the time was that the Pharmacology Department under Allan Bass' leadership was willing to think about recruiting a basic person into the Department of Pharmacology to ally with the Clinical Pharmacology research interests. I don't think there were many other pharmacology chairs in the country who would have put a tenure-track position on the line to support clinical pharmacology in that way. It was a fairly unique decision both with Grant and Jack Watson, both of whom had had tenure track positions in pharmacology. Other pharmacology leaders around the country had a much more narrowly constricted view of what a pharmacology department ought to be.

JDM: Okay. With that I think we'll stop.