Whole animal physiology redux

EVEN THOUGH I RETIRED COMPLETELY in 1998 and vowed never to attend another meeting, I had to renege because it is such a great honor to be invited to give this lecture honoring my mentor, Julius Comroe. I thank the selection committee for considering me. Of the nine scientists chosen since this lecture was established, I may be the first unreconstructed, whole animal physiologist.

Before I left San Francisco for New Orleans, I received one of the abstract volumes for Experimental Biology 2002. On the airplane and in my hotel room, I studied the pulmonary abstracts, of which I identified about 115. I made a list of the species used in the investigations, including 30 rats; 24 humans; 14 mice; 11 dogs; 4 rabbits; 3 each cats, rabbits, and goats; but only 1 sheep. Ten years ago, I would have expected 20 abstracts using sheep.

Not only has the distribution of species changed, but intact animals were not used in many of the studies. For example, you might think that 24 experiments involving humans is encouraging, but in most reports, the humans were used to obtain populations of cells or proteins by lung lavage or from blood samples. Those do not qualify as integrative biological studies, in my opinion.

A serious malaise has overtaken physiology. Physiology has become so bedazzled by molecular biology that physiologists are not producing new physiological insights. I have nothing against petri dish or test tube physiology. I have done many such studies. In its place, it is useful. What I decry is not the rise of mouse physiology but the decrease of whole animal physiology. So, I have been considering what I should say today that might make a useful contribution to physiology, the queen and mother of biological sciences. I’ve decided that the overall theme of my talk will be a question: Why has the potential for applications of molecular biology in clinical medicine not been realized?

In 1990, I took a sabbatical to learn more about macrophage biology and molecular research. I learned many useful techniques. I even did some experiments to demonstrate that macrophages selectively phagocytize apoptotic leukocytes. But the most important thing I learned was that it is not my kind of research. I am too impatient to wait for a macrophage to ingest an old leukocyte, and I find reading blots boring. I need to record and measure things as I go along, antiquated physiological measures such as lung lymph flow, body temperature, pulmonary arterial blood pressure, and cardiac output.

In 1993, the year I officially retired, I received a small grant from the American Heart Association (AHA). The award was due almost entirely to my longtime friend, Prof. Aubrey Taylor of the University of South Alabama, who lobbied on my behalf. The next year, the AHA changed its rules, so that old, unreconstructed physiologists would not be eligible for grants. I spent the next four years in the laboratory working with Dr. Kim Longworth at the University of California Davis.

This brings me to what I believe is the problem behind my theme question (Fig. 1). There is a disconnection between molecular biology and clinical medicine because whole animal physiology has been downplayed, sidetracked. Despite fundamental discoveries, there is an enormous gap separating molecular biology from the treatment of disease. Few of the touted applications work. Some have unexpected, even dangerous consequences. That’s some disconnection! I believe the gap can be bridged if physiologists lobby and promote

---

Address for reprint requests and other correspondence: N. C. Staub, PO Box 965, Stinson Beach, CA 94970.

http://www.ajplung.org 1040-0605/02 $5.00 Copyright © 2002 the American Physiological Society

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
whole animal experimentation and learn to collaborate with naive molecular biologists to do relevant research in large animals suitably instrumented. The war on lung cancer, declared in 1971 by President Nixon, has not been won. Asthma is more prevalent than ever, and acute respiratory distress syndrome is still very much with us, although there has been considerable progress.

JULIUS COMROE’S LEGACY

Julius Comroe was raised in Pennsylvania and did his medical studies at the University of Pennsylvania. After graduating in 1934, he planned to become a surgeon. However, he was thwarted when, as an intern, he lost an eye that had become infected while he was treating a patient. He had to settle for a career in teaching and research. Even though Julius did excellent original work on arterial chemoreceptors and the control of breathing, I believe his legacy rests on his inspired and innovative teaching and his gift for organization.

I will describe one of his earliest innovative ideas. In 1946, that is, in the first half of the last century before most of you were born, there was a great influx of World War II veterans to universities. Many were doctors who had served in the military for several years. They wanted specialty training but had been out of touch with advances in the basic sciences. Dr. Comroe proposed a course to meet their needs. He worked with other faculty members in the University of Pennsylvania’s Graduate School of Medicine to plan an integrated refresher course in the basic medical sciences. That refresher course became nationally and internationally famous for the next decade.

Also in 1946, Dr. Comroe organized the Department of Physiology and Pharmacology in the Graduate School of Medicine (University of Pennsylvania). He began to apply some of the wartime technological developments to lung physiology. He and his associates made a landmark contribution when the first edition of The Lung appeared in 1955. That book marked the beginning of modern pulmonary physiology and chest medicine. Early on, Dr. Comroe had the idea for a body plethysmograph. His young colleagues, notably Arthur Dubois, made it practical and applied it to a wide array of pulmonary problems. When I came to the Department of Physiology and Pharmacology in 1956, I knew little about research. Dr. Comroe suggested that I work on the body plethysmograph. I had no idea what that was. But it never happened because he shunted me into his new, experimental Medical Faculty Training Program.

In 1957, Dr. Comroe moved to a small, provincial medical school in California, now the University of California San Francisco. He went there to be recruited as chairman of the Department of Pharmacology. However, on a tour of the new teaching hospital under construction, he saw all of the empty space on the 13th floor that had been reserved for the Cardiovascular Research Institute (CVRI). He asked to be considered for the director’s job. When Dr. Comroe decided he wanted something, he could be very persuasive. So, he got the job. The CVRI officially opened on October 30, 1958. Dr. Comroe was also appointed as Professor of Physiology. Each of the full-time faculty in the department was a whole animal physiologist, more or less. Three of the faculty members became future American Physiological Society (APS) presidents: Dr. Comroe, 1960; Dr. William Francis Ganong, 1977; and myself in 1991.

The CVRI started small but got really big, really fast. Shortly after arriving in San Francisco, Dr. Comroe met Dr. Glenn Seaborg, Chairman of the mammoth, Nobel Prize-riddled Department of Physics at University of California Berkeley. He asked Dr. Seaborg why his department received so many Nobel Prizes. Dr. Seaborg replied that it was because his department was the biggest in the world. Dr. Comroe liked that idea, and he set out to build the CVRI into the largest organized research unit in the world. During a visit to the CVRI in 1961, Dr. James Shannon, the enlightened first director of the National Institutes of Health (NIH), suggested to Dr. Comroe that he merge the new Institute’s individual NIH awards into one center grant. Dr. Comroe planned and largely wrote the first Program Project grant. Dr. Shannon gave him practically everything he requested for seven years. Later, it was renewed for another seven years. I told you Dr. Comroe could be very persuasive.

Many years later, when reviewing the early days of the CVRI, Dr. Comroe wrote, “Those were the good old days when the director and associate directors of the NIH said, ‘What can we do to help you?’ instead of ‘We regret to inform you . . . .’” To build his Institute, Dr. Comroe sacrificed his research career. He believed it was a full-time job to provide the leadership, to create an atmosphere of collegiality, and to procure the space and money for the Institute’s growth so that the scientific staff and fellows could do their research.

Dr. Comroe was a great organizer of research. Never forgetting that he was spending the taxpayers’ money, he believed in three principles for efficient research: 1) select intelligent, promising investigators; 2) give them money and space; and 3) let them do research without interference from the top. Dr. Comroe did not trust top-down planning. He did not believe that either government or volunteer health agencies should tell scientists what to discover. Some years later, in his Retrospectroscope essays, he delighted in demonstrating that many important discoveries in medical science happened by chance.

Around 1970, Dr. Comroe and his longtime colleague Dr. Robert Dripps, Chairman of Anesthesia, University of Pennsylvania, began a formal research project that aimed to quantify how important advances in clinical medicine came about. They published their results in Science in 1976. Using 10 widely accepted major advances in the understanding and treatment of cardiovascular disease, they demonstrated that >40% of the key discoveries leading to successful clinical applications of basic research were made accidentally,
that is, by observant scientists working on something else. Their paper was hailed far and wide! The NIH gave the Comroe and Dripps paper lip service but generally ignored it. By then, the NIH was enthralled by the concept of strategic planning, a grandiose scheme to direct the funding of research from the top down. Sadly, Dr. Comroe died in 1984 after a long battle with metastatic prostate cancer.

DETERGENT ATTENUATES ACUTE RESPONSES TO ENDOTOXEMIA IN SHEEP

In this section, I present some data concerning a well-defined pathophysiological problem: how to explain the acute pulmonary vascular responses after the intravenous infusion of small quantities of endotoxin in sheep.

The primary cause of the sheep’s pulmonary response to endotoxin is the stimulation of a resident population of macrophages in the pulmonary capillaries. The evidence for that statement is based on experiments using whole animals: newborn, juvenile, and adult. It is a problem in integrative biology and demands answers based on whole animal investigation. Now that the main work of discovery has been done, it seems logical that molecular and cellular biologists will work on the details and make important contributions toward understanding the mechanism.

More than 75 years ago, investigators reported that different mammalian species showed widely different responses to the intravenous infusion of particulate matter. Such differences could not be readily explained. Some of the speculations offered included activation of phagocytosis by lung capillary endothelial cells, activation of circulating monocytes or other leukocytes, or the migration of liver macrophages to the lung. Although my associates and I noted occasional pulmonary pressor responses to various infused substances, I did not recognize the significance of the problem until the early 1980s, when my colleague Dr. Kurt Albertine was trying to localize the sites of protein leakage in the pulmonary microcirculation of anesthetized sheep. He was bothered by the acute rises in pulmonary arterial pressure that occurred whenever he infused small quantities of particulate tracers intravenously, tracers that caused no reaction in most species of laboratory animals.

Back in the 1980s, I made a graph by plotting the mean lethal dose of intravenous endotoxin in several species. In those days, such data were readily available in the literature, when survival was often used as the experimental end point. The species can be readily divided into two groups: a large group that includes most laboratory animals that are resistant to death by endotoxin and a small group that is 100–1,000 times more sensitive. What makes the division among mammals interesting is that the sensitive species are those that possess a large population of macrophages permanently adherent to the endothelium of the pulmonary capillaries. These resident macrophages were conclusively demonstrated in the lungs of cattle in 1974.

We labeled the macrophages by infusing Monastral blue pigment particles. The macrophages are readily identified by the bright blue particles of Monastral blue pigment that they have phagocytized. These cells are so numerous and so phagocytic that they remove almost all of an intravenous particulate load during the first pass. At autopsy, the lungs are bright blue after the residual blood has been washed out.

On electron microscopy, macrophages are attached to the endothelium of sheep and goat lung capillaries by focal adhesion plaques that attach the macrophage permanently to the endothelium. As physiologists, you’ll no doubt recall that all mammals have a large population of phagocytic macrophages in the lung capillaries, is not known. By the way, the endotoxin-sensitive species appear to be limited to two orders of mammals, those with an even number of toes (artiodactyla; sheep, pig, goat) and those with a single toe (perissodactyla; horse, pony).

In the sheep and other endotoxin-sensitive species, some monocytes remain adherent to the pulmonary capillary endothelium and mature into resident intravascular macrophages. When I was active in research, I thought working out the mechanism for the macrophage attachment would be an ideal model for a molecular biologist interested in adhesion molecules. However, it engendered essentially no interest whatsoever. Apparently, working with any animal larger than a mouse scares molecular biologists away. But the same type of focal adhesion plaques binds the liver macrophages to the sinusoids. One could use mouse liver to study the adhesion problem.

In 1988, I met Dr. Vladimir Serikov of Leningrad. He told me about some experiments he had done using detergents to study transcapillary protein transit in the lungs of anesthetized dogs. I was so impressed by his unique ideas and provocative experiments that I invited him to come to San Francisco with his family. One day he showed me that the addition of a detergent to the blood perfusing a sheep lung blocked the expected rise in pulmonary arterial pressure that always occurs after a standardized infusion of Monastral blue pigment particles.

We decided to test the effects of the biosafe detergent tyloxapol on the response of the intravascular macrophages to endotoxemia of intact sheep. This is what we did during that AHA grant I mentioned earlier. Fortunately, tyloxapol is very safe detergent compared with its parent molecule Triton X-100. Thus we were able to give successive doses of tyloxapol up to a cumulative dose of 150 mg/kg (~30 μmol/kg) without causing hemolysis or any other detectable side effect (Fig. 2).

After a 2-h baseline, Dr. Kim Longworth and I infused 1 μg/kg of *Escherichia coli*, which is a tiny dose, considering that cell physiologists routinely add 1–10 μg/ml to their petri dish experiments. For this research design, the control experiment using endotoxin alone
was done first and the detergent experiment a week later. In the control study, body temperature increased by the end of the infusion and continued to rise to a peak of about 42°C; the circulating leukocyte concentration decreased to a low level over 2 h; there was a sharp rise in pulmonary arterial pressure during the endotoxin infusion, and the lung lymph protein clearance (which is a convenient index of microvascular permeability to protein) rose steadily, reaching a plateau about five times the baseline level by the third hour (Fig. 2). We did a total of 10 sheep studies. The results confirm the marked attenuation of all measured responses after tyloxapol (Fig. 3).

We had tried unsuccessfully for two years to develop a bioassay for sheep tumor necrosis factor-α (TNF-α). Fortunately, I learned that Dr. Ted Elsasser, Department Agriculture Laboratories, Beltsville, MD, had a successful radioimmunoassay for sheep TNF-α. He agreed to join our team and to measure the TNF levels in plasma and lymph. As you might expect from the temperature response in the control study, shortly after the endotoxin infusion was completed, the circulat-

![Fig. 2](image-url)  
*Fig. 2. Summary of time course of 4 variables during 2 experiments in each of 10 awake, instrumented sheep studied 1 wk apart. From top to bottom, the y-axes show lymph protein clearance (A), mean pulmonary arterial pressure (B), circulating leukocyte concentration (C), and body temperature (D). Value bars are means ± SE. Compared with the control study (dashed lines), the effect of endotoxin on all variables is markedly attenuated after tyloxapol treatment (solid lines). *P < 0.05 between the data pairs; the majority of P values were <0.001 (1).*

![Fig. 3](image-url)  
*Fig. 3. Summary of time course of plasma and lymph TNF-α concentrations in 10 awake, instrumented sheep for 2 experiments each. Dashed lines are endotoxin alone (control); solid lines are endotoxin after tyloxapol. Tyloxapol inhibited the circulation concentration of TNF by >90%. *Differences between groups are significant (P < 0.001) at all times after endotoxin (1).*
ing plasma TNF-α concentration increased to a high level, accompanied by a smaller rise in the lymph protein clearance. Tyloxapol substantially attenuated the TNF-α response (Fig. 3).

In summary, in the control experiment using endotoxin alone, there was a large rise in the plasma TNF-α concentration for ~2 h. The lymph TNF concentration increased but lagged behind that of plasma and never reached the same peak concentration. Therefore, I concluded that the source of the TNF-α was the intravascular macrophages. After tyloxapol, the rise in plasma and lymph TNF-α concentrations was markedly attenuated.

Dr. Serikov and I believe that tyloxapol acts directly on macrophages to block one or more macrophage receptors, which decreases their ability to synthesize or secrete prostanoids and cytokines. How this occurs is not known. One surfactant chemist suggested that tyloxapol coats the surface of macrophages, thereby blocking access of endotoxin to its receptors in the same manner as tyloxapol binds to the surface of endothelial cells to block the access of LDLs to lipoprotein lipase. It must be a fairly strong binding because the cells are inhibited for >1 wk.

A RENAISSANCE FOR WHOLE ANIMAL PHYSIOLOGY

Remember my basic theme question in the introduction: Why has the potential for applications of molecular biology to clinical medicine not been realized?

Perhaps Fig. 1, DISCONNECTION, is corny, but it shows the Clinical Medicine Caboose sidetracked as the Molecular Biology Train speeds off. I will not pursue why this occurred. There is plenty of blame to go around. I also used an example from my recent research to give a glimpse of basic and applied research, using whole animals in a manner that I thought might appeal to you. So, I will end by making the following assertion: Whole animal physiology is desperately needed to facilitate the reconnection between clinical medicine and molecular biology (Fig. 4).

You may recall that in the 1980s, there were frequent teaching sessions by molecular biologists at the APS and Experimental Biology meetings. These served to awaken physiologists to the potential for application of the ideas and tools of this new discipline in their research with whole animals. Taking a leaf from their successful scheme, I propose that the APS introduce a new type of teaching session at scientific meetings. Yes, I am suggesting that you become missionaries on behalf of whole animal physiology. The overall aim of these new sessions should be to awaken an interest in physiology of molecular biologists by demonstrating the heritage and success of whole animal physiologists dealing with real problems of humankind. Each session ought to include one or more examples of how whole animal physiology played a key role in reconnecting molecular biology to clinical medicine. Any area of physiology could and should be used; the specific problem is not as important as the concept that integrative biological studies are vital to the successful application of molecular biology to clinical medicine. The teachers should be active physiologists, who are able to speak clearly and avoid jargon and excessive detail. I hope they will be knowledgeable across the spectrum of molecular biology to physiology to clinical medicine. Finally, I recommend that the Respiration Section assume the leadership in promoting this renaissance in whole animal physiology.

REFERENCES


Norman C. Staub, Sr.
Professor of Physiology Emeritus
Cardiovascular Research Institute
University of California
San Francisco, California
October 2002, Volume 283 (28)