‘COMMUNICATION’ IN SCIENCE

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In recent years, the term “signal” has become popular concomitantly with the contemporary concept of an information-oriented age. However, for those of us who are engaged in medical research, the scientific concept of a signal is by no means a novel or extraordinary one, since signal-related substances such as hormones or neurotransmitters have been a long standing important topic in our research, and the emergence of scientific studies on the mechanism of action of these substances is not an especially new development.

The human body is said to be formed from approximately 50 to 100 trillion cells. That is a truly astounding number! The 50 trillion or more cells constituting our bodies do not function separately and independently, rather, they act with magnificent mutual coordination. For example, in the action of the brain and the movements of the limbs and in the secretion of saliva and the motion of the digestive tract—in fact, everywhere in the body—one can observe amazing coordination of cellular activities. Numerous hormones, neurotransmitters, growth factors, and many other physiologically active substances function so as to permit the smooth implementation of these coordinated cellular activities.

As is well known, hormones are secreted by endocrine cells and govern the functions of their target cells via transport by the blood stream (Figure 1). On the other hand, as regards neurotransmitters, extremely long processes issue from neurons, and in fact if a neuron cell body were the size of this conference hall in Kyoto, then the terminals of the axon would extend as far as Aomori or even to Sapporo in Hokkaido. However, like an electrical current, impulses are transmitted almost instantaneously and cause the rapid emergence of chemical substances at Sapporo, say, thereby conveying the required signal. Also, the term “local hormones” is written here, which signifies that cells of the same or different types cohering in tissues and organs emit various chemical substances, thus conducting what might be compared with a conversation and thereby

accomplishing mutual adjustment of functions.

The stream of research directed toward the elucidation of these mechanisms can apparently be divided into two major categories.

Firstly, research in this area conducted in the United States was initiated in St. Louis more than 50 years ago. At that time, Washington University was founded in St. Louis, and two professors from Europe, named Carl Cori and Gerty Cori, were enrolled in the faculty of the new university, and devoted their efforts to the establishment of the new medical school. Professor Carl Cori was interested in the regulation of blood sugar levels by the hormone adrenalin. When we are exposed to cold, or feel hungry, adrenalin is secreted by the adrenal medulla, acts upon the liver, and thereby causes the rapid breakdown of glycogen into glucose, which enters the bloodstream and is utilized as fuel. On the other hand, Professor Gerty Cori was interested in the mechanism of action of insulin, which manifests the reverse effect. Thus, the present area of research commenced from the work of these two scholars on the regulatory mechanism of action of glycogen metabolism.

After having eaten, during our nocturnal sleep, the excess energy in our bodies is stored in the liver in the form of glycogen. However, if adrenalin arrives, then glycogen is promptly decomposed and enters the bloodstream in the form of glucose. The first problem in this connection was to clarify the mechanism of this phenomenon. Proper regulation would be impossible if glycogen were constantly degraded throughout the day and night. That is, glycogen degradation occurs only upon the arrival of adrenalin. This appears patently obvious, but the difficulty in truly comprehending apparently obvious phenomena constitutes the charm and fascination of the life sciences!

Today, I shall omit unduly complicated explanations. The first enzyme which degrades glucose is known as glycogen phosphorylase (Figure 2). Simply stated, this enzyme ordinarily exists in an inactive form, but upon the appearance of adrenalin is immediately converted to an active form, and thus degrades glycogen. When adrenalin appears, glycogen is degraded instantaneously, or more precisely, perhaps, in just a few seconds, and therefore a previously absent enzyme presumably could not be synthesized from the its constituent amino acids in time to effect this degradation.

At present telephone or facsimile equipment permits immediate

communication with the entire world, whereas a letter placed in a mailbox may not arrive in time. The enzyme under consideration originally exists in an inactive form, but upon the arrival of adrenalin, in accordance with an ingenious mechanism, the phosphate groups of ATP bind to the inactive form of the enzyme, and the activity of the enzyme is thereby instantaneously manifested. If adrenalin ceases to arrive, that is, if an energy source becomes unnecessary, then, by the action of another enzyme, called phosphatase, these phosphate groups are removed, and the enzyme returns to the original inactive form. That is, the mechanism is such that the inactive and active forms of the enzyme can be instantaneously and reversibly converted to one another as needed.

This is an extremely simple explanation, but the simpler a phenomenon, the more elusive the explanation may be. In fact, the elucidation of the present phenomenon required well over a decade. The previously mentioned Professors Cori and Cori, at any rate, succeeded in extracting this enzyme, and established that this was indeed the enzyme which breaks down glycogen. This occurred 50 years ago, in 1934, and by virtue of this discovery Professors Cori and Cori received the Nobel Prize in Medicine in 1947. At that time, they had noticed that the enzyme existed in both active and inactive forms, but the mechanism of conversion was completely obscure.

This mechanism is actually extremely simple; as I mentioned previously, when the phosphate group of ATP are bound, the enzyme assumes the active form, and conversely, if the phosphate groups are removed, then it reverts to the inactive form. The fact that the two forms are interconverted in this manner was discovered during 1950s decade by Professor Ed Krebs, a disciple of Professors Cori and Cori, who had originally worked in the latter's research group, and by Professor Eddy Fischer of Geneva University in Switzerland, who joined with this research. The discovery was announced from Seattle in 1955, and was rewarded by the Nobel Prize in Medicine for the present year. Thus, the research involved was conducted 40 years ago. This research was not conducted at Washington University in St. Louis but rather at Washington University in Seattle.

In any case, one of the problems involved at that time was that although adrenalin is transported from the adrenals and arrives at the liver, it does not enter the liver cells in question. Nevertheless adrenalin regulates the intracellular event under

consideration. What, then, is the explanation for this phenomenon? That was the problem to be resolved. The researcher who pondered over this problem was Professor Sutherland, who also had studied in Professor Cori's group and as a student had been two years senior to the aforementioned Professor Krebs. Through his research on this mechanism, Professor Sutherland, in 1958 arrived at his celebrated discovery of cyclic AMP. Again, this discovery was subsequently recognized by the award of the Nobel Prize in Medicine in 1971. Thus, this same research topic, the degradation of glycogen, initiated by Professors Cori and Cori more than 50 years ago, has—if his disciples are included— been recognized by the award of three Nobel Prizes, including that for the present year.

The topic of my address today is related to human encounters in the realm of science, but in the final analysis it actually concerns the vital importance of human propagation of learning. Research is not something suddenly created on some particular day, and I wish to emphasize that any significant research result actually possesses a long history extending over several decades.

Returning to our original subject, cyclic AMP is actually an extremely simple compound. According to the novel hypothesis expounded by Professor Sutherland, liver cells are stimulated by adrenalin, whereupon cyclic AMP is formed within the cells, and this substance performs various cellular functions in place of adrenalin. However, theories which subsequently undergo further extension and development usually are novel and original, while answers to problems accessible to resolution by a straightforward rational approach can generally be anticipated by almost anyone, and therefore constitute no particular grounds for selfsatisfaction.

This hypothesis of cyclic AMP did not attract especially great attention at the time. However, truly incisive research will inevitably be noticed by someone in this world. In the present case, researchers in related fields have further developed this theory from various viewpoints. The field of view of a single researcher is ordinarily rather narrow and inevitably limited. Therefore, proximity to a large number of other researchers is of vital importance. Thus, encounters among scholars scrutinizing nature from a variety of different viewpoints enlighten the subjects under consideration and promote the progress of research.

The next problem was that of explaining the mechanism whereby this simple

substance, cyclic AMP, can function in place of adrenalin. This problem was resolved by the aforementioned Professor Krebs, who found a novel enzyme, that is cyclic AMP-dependent protein kinase, now formed protein kinase A. This enzyme catalyses phosphorylation of many proteins in the presence of cyclic AMP, and leads the inactive glycogen phosphorylase to it active form. In fact this resolution was a natural consequence of the line of research which he had long pursued. This occurred 25 years ago. The mechanism of action of cyclic AMP is schematically illustrated in Figure 3. The right-hand side of the diagram can be regarded as representing a cell, but arriving hormones do not actually enter the cell. The stimulus of the hormone is recognized and perceived at a receptor on the cell membrane, which constitutes antennae, so to speak, for the incoming signal, and by virtue of this signal, cyclic AMP is formed from ATP by the catalytic action of a certain specific enzyme on the cell membrane. This enzyme was also found by Professor Sutherland and on the basis of this discovery he proposed the hypothesis that cyclic AMP acts as an intracellular second message for the degradation of glycogen. This hypothesis was subsequently extended by various other researchers. That was roughly the period around 1968, when I first assumed a post at Kobe University.

Excited by this research, our group commenced studies on protein phosphorylation reactions. Just at that time, the clock tower of Tokyo University had been inundated by water from a hose during a period of campus turmoil, the libraries of nearly all the universities in Japan were closed, and water, electricity and gas supplies to our own research facilities had been suspended. At that time, only the Kobe University library was open, and I read the report published by Professor Krebs describing how cyclic AMP activates glycogen phosphorylase. Namely, he discovered an enzyme which can phosphorylate proteins in the presence of cyclic AMP. The fact that phosphate was covalently bound to protein was previously the subject of research by Professor Lipmann, who was my own teacher during my period of postdoctoral fellow at the Rockefeller University in New York, and received the Nobel Prize in 1953. Therefore I was profoundly interested in protein phosphorylation reactions.

During the 1970s, prevailing opinion held that the mechanism of action of hormones could be almost completely explained by the line of research which had been continued and propagated by Professors Cori and Cori, Sutherland, Krebs and Fischer.

The research on cyclic AMP became extremely popular in many academic circles.

However, nature is actually not so simple. At that time a doubt arose in my mind, because I knew that calcium ions are indispensable for the action of many hormones; this concept had been established by European and particularly British researchers since the previous century. However, as you can observe from the diagram in Figure 3, calcium ions are not involved in any phase of the putative mechanism of hormonal action depicted here. Hence, I vaguely felt that some other major mechanism might be involved in the actions of many other hormones. In fact, omitting the details, I finally realized that, in addition to protein kinase A, another enzyme, one requiring calcium ions, plays crucial roles in hormone actions. In addition to the great efforts of my numerous collaborators, many scientists both within and outside of Japan also contributed greatly to the development of this story.

I have previously alluded to the two major historical background factors relating to research on the mechanism of hormone action. One of these is the history of more than 50 years of research in the United States which I have just mentioned. The other is the relevant research conducted in Europe, which likewise has an extremely long history of more than five decades. In particular, the researches conducted in Britain, Belgium, and the Netherlands are fundamental to the subject. In general European scientists are decidedly not prone to be infatuated by current fashions. I suppose this characteristic is attributable to various factors, but it is primarily due to the European scientific history and tradition of several hundred year's duration.

During that period, I had an opportunity in Brussels of meeting Professor Gery Hers. Professor Hers was then considering the same line of research as myself. Some aspects of adrenalin or neurotransmitter activity are, perhaps, explicable in terms of cyclic AMP. However, as exemplified in Figure 4, the action of a large number of hormones or neurotransmitters and other biologically active substances may not involve cyclic AMP. Such action mechanisms inevitably require calcium ions.

Professor Hers had also been studying the degradation of glycogen and regulation of blood glucose levels, and at present is still in Brussels. Hormones such as angiotensin and vasopressin also act upon the liver and cause the breakdown of glycogen. However, extensive investigations failed to reveal any elevation of cyclic AMP content in the liver during the action of these hormones; and the activity of the

enzyme protein kinase A, discovered by Professor Krebs, did not display any elevation. The fact that calcium ions are absolutely necessary for the action of angiotensin and vasopressin had been observed long before. Therefore, Professor Hers also considered that a more general mechanism, not involving cyclic AMP, governs the functions of many biologically active substances. This meeting constituted a source of great encouragement to me. I commenced to devote my efforts toward research in that direction.

The research conducted by our group was not principally concerned with cyclic AMP, but with cell membranes. About the year 1950, a young couple named Mabel Hokim and Low Hokim were studying for their degrees in physiology at Sheffield University in England. As is well known, the pancreas secretes a digestive juice, and this function is controlled by acetylcholine. If a slice section of pancreatic tissue is immersed in Ringer's solution and warmed at a temperature of 37 degrees, and acetylcholine is added to the solution, digestive juice effuses from the tissue specimen. Their research was concerned with the mechanism of this phenomenon. Radioactive isotopes were first applied to medical research in Britain. At Sheffield University, they managed to obtain isotope materials, and using these they noticed that if pancreatic cells are exposed to acetylcholine, then a certain constituent of the cell membrane undergoes an extremely rapid cycle of synthesis and degradation.

However, job opportunities in Britain then were limited, and many outstanding scholars were emigrating to North America. The Hokims were among this number. They assumed posts at McGill University in Canada in about the year 1953.

Cell membranes are composed of two superimposed thin oily layers known as a "lipid bilayer." These oily substances are known as phospholipids. In Figure 5 we have a series of heads arranged in row. Although not drawn here, another row of heads is arranged on the lower side, facing the opposite direction. That is, two layers are present. Two "legs," so to speak, grow from each head. These "legs" represent fatty acids. The region above the heads represents the exterior of the cell, and that below the heads the interior. The fatty acid portion is insoluble in water, and since oily materials such as fatty acid repel water and attract one another, the "legs" cling together and thereby separate the exterior and interior of the cell. The heads are the portions which are readily soluble in water, or in more specialized terminology, the "strongly polar"

moieties. Thus, the basic structure of the cell membrane consists of hydrophilic heads facing the water side and hydrophobic oil aggregating the legs, thereby forming a partition. The “head” portions are of four types, that is, serine, ethanolamine, choline, and inositol. The previously mentioned Mabel and Low, after intensive research at McGill University, discovered that not all of the four types of heads, but only inositol, represented by the head with the derby hat, is ejected when acetylcholine arrives, and that after several minutes this disruption is repaired and the inositol returns to its original position. However, at that time, they did not comprehend the significance of this phenomenon.

I have previously mentioned calcium ions; in fact, the calcium concentration on the exteriors of the 50 trillion cells composing the human body is extremely high. However, although I use the word “high,” this is not of the magnitude which we ordinarily consider, and is actually of the order of 1/10,000 molar. However, the intracellular calcium concentration is several thousand times more dilute, being of the order of 10^{-7} molar. The entry of even a minute quantity of calcium ions from the exterior of the cell will trigger the activation of a variety of intracellular processes. This concept has been established by British physiologists through more than a century of assiduous research.

Physiology originally commenced from the study of muscular contraction. The water in the districts of London, Cambridge, and Oxford is hard water, with a high content of calcium ions. A phenomenon not noticed prior to the use of distilled water and discovered only when distilled water came into use for laboratory work during the 19th century is the fact that muscular contraction does not occur in the absence of calcium ions.

The relation between the requirement for calcium ions and the ejection of the “head with the derby hat” was not understood by Mabel and Low at that time. However, this phenomenon is not confined to the action of acetylcholine. Many researchers in various parts of the world have reported that the “head with the derby hat” is also ejected by the action of a great many other hormones and neurotransmitters.

Thus, consideration of these research results naturally suggests the possibility that the ejection of this head may be related to calcium entry into the cell. In other words, the calcium ion concentration outside the cell is high, while that within the cell is

low, and when the head is ejected, the headless “torso” remains within the cell membrane, but an aperture is created. Thus, an influx of calcium ions from the exterior could trigger the activation of various intracellular functions.

This hypothesis was considered by one scientist, namely, Bob Michell, who was at Nottingham University in England at that time. Bob is a good friend of mine, now about 53 or 54 years of age, a fellow of the Royal Society, sports a superb beard, and always presents the appearance of a hippie, wearing a headband. In 1975, that is, about 16 or 17 years ago, Bob, having read a great deal of literature on the subject, proposed a new brilliant hypothesis. That is, when a hormone arrives from the exterior, the head with the “derby hat” is ejected, and this permits the entry of calcium ions from the exterior, which perform certain intracellular functions. He discussed that hypothesis with his supervisor Professor Tim Hawthorne, who, incidentally, I also greatly admire. However, Professor Hawthorne was severely critical, and questioned the evidence for the hypothesis. It is true that, in science, speculation is unrestricted, but that any assertion should be supported by at least a minimal quantity of experimental evidence. Bob humbly and honestly accepted this criticism, and continued his researches, but still could not manage to secure the desired evidence. Nevertheless, having obtained the understanding and approval of Professor Hawthorne, he published an article in 1975 stating his hypothesis in a learned journal. This article aroused considerable interest, and the reason for the ejection of the head with the “derby hat” became the subject of renewed discussion.

I shall omit the details, but in 1977, our group first discovered an enzyme in the brain phosphorylating proteins in the presence of calcium ions, and published a brief article concerning this fact. I received a letter from Professor Hawthorne who, I believe, served as the Vice-Chancellor of Nottingham University until just recently. Although I had not previously met him, I was invited to attend a certain conference at Nottingham for the memory of Professor Folch.

The conference was held in an ordinary university lecture hall, and I mentioned the following notion. That is, after the head has been ejected from the cell membrane, the headless torso remains and a completely new protein phosphorylating enzyme, present in large quantities, proceeds to function. Since English is not my forte, I spoke in a low-pitched manner, and in any case I was seated at the very rear of the lecture hall.

After the conclusion of the meeting, Professor Hawthorne conducted me to the university bar and poured me a glass of beer. There were two or three young persons present, but we were shortly joined by four or five fairly elderly scientists. I was informed that the research I was conducting was in fact related to concepts which they themselves had been considering for several decades, and they expressed their strong support and encouragement for the work of our group. I shall not cite the individual names of these scientists, but this also constituted a most rewarding encounter. On that occasion, I was enlightened by those scientists concerning the properties of various types of lipids.

During my return flight, I considered various possibilities, but of course mere consideration provides no proof. If a hormone arrives at the cell membrane, then an appropriate “antenna” or receptor there will be acted upon and eject the head. If a headless torso remains, then the activity of the enzyme (Protein kinase C) is manifested, thereby regulating various cellular functions. However, this so-called headless torso (diglyceride) is an oily substance, and if applied externally does not enter the cell. That is, being insoluble in water, this substance does not penetrate the membrane. In our research group, we were considering whether there existed a method whereby this “headless torso” could somehow be thrust from the exterior into the cell in order to observe the resulting effects. Now, this so-called “headless torso” possesses two “legs.” I then conceived the notion that if one of the legs was shortened, then the properties of soap would be imparted to that substance. In the process of arriving at this notion, the ideas not only of overseas scientists but also of the young researchers in my laboratory had been highly suggestive. A soap is formed by a single fatty acid, that is, it has just one leg. Realizing that such a molecule would penetrate the cell membrane to a certain extent, I was confronted by the problem of synthesizing a “headless torso” with a short leg, but unfortunately my proficiency in chemistry was limited. Fortunately, however, I happened to meet Dr. Hiroo Maeno, a former junior high school classmate, who had studied chemistry at Nagoya University, and is now a pharmaceutical company director.

I asked if he could prepare the short-legged substance under consideration, whereupon the substance was promptly synthesized for us by Hiroo together with his colleagues. This substance did indeed directly enter the cell and was found to activate the enzyme in question, that is protein kinase C. I shall forego the details, but from the

exterior of the cell and without destroying the cell, we were able to activate the functions of this enzyme, which corresponds to a sort of signal amplifier within the cell.

During that period, another significant encounter occurred. This was a meeting with a French researcher named Monique Castaqna. I received a letter May 1981. A famous cancer research institute is located at Villejuif directly south of Paris; “Ville” means “village”, while “juif” apparently means “Jewish.” The letter had been sent from that institute, and was extremely simple, consisting of merely about three lines, stating that the writer wished to visit Kobe for a period of one month in August, and mentioned a previous meeting with me in Belgium. Even a short visit of about one month in August would be subject to somewhat unfavorable conditions, such as hot weather and summer vacation, but after consultation with my colleagues I somewhat reluctantly agreed to the request.

Subsequently, I was informed that the correspondent was scheduled to arrive at Osaka Airport on August 1st. I sent a graduate student to meet the visitor, and promptly received a telephone call. “Professor, the visitor is a lady,” I was informed. I had assumed that the visitor would be male. I instructed the graduate student to arrange a room for the visitor at the Portopia Hotel. On the following day, August 2nd, since the visitor was a lady from Paris, I scrupulously donned a proper business suit despite the heat; and when I met the lady, she spoke volubly in English with a thick French accent, and I experienced some difficulty in understanding. Likewise, I spoke English with a thick Japanese accent, which was equally incomprehensible to her. At that time, this lady asked whether I was familiar with phorbol esters (tumor promoters), and I replied that I had no detailed knowledge of the subject. Retrospectively, although somewhat ashamed of my ignorance, I now considered that this incident was most fortunate. I could not very well have glibly feigned knowledge of the subject, but fortunately, if one confesses ignorance, then unlike Japanese professors, foreign scientists will earnestly and cordially explain the subject in question.

Monique unexpectedly extracted a voluminous photocopy from her briefcase, and suggested that I read the photocopy and excused herself. This photocopy was a review article concerning the promotion of carcinogenesis, written by Peter Blumberg, who was then at Harvard University, and subsequently also became a close friend of mine.

First, I shall briefly explain the nature of these "tumor promoters." The croton plant is a fruit bearing shrub which grows in certain subtropical regions; the Japanese name of the plant is "*hazu.*" The croton plant has been used in China since antiquity as a potent purgative in the traditional Chinese pharmacopoeia. Some of you in the audience probably also remember, but I recollect that in my generation, during the Second World War, after having eaten some spoiled food, we received an oral does of castor oil as a purgative. Croton oil is probably similar in this respect. The oil expressed from croton seeds is a powerful irritant and has been involved in research on carcinogenesis. As some of you probably know, research on chemical carcinogenesis was initiated by the Japanese scientist, Professor Katsusaburo Yamagiwa, whose research achievements constitute a justifiable source of pride for Japanese biological scientists. Professor Yamagiwa studied in Germany, and his researches commenced with studies of the skin cancer which constituted an occupational disease of chimney-sweeps. After returning to Japan, acting upon the hint provided by the soot which accumulates in chimneys, he daubed coal tar onto the ears of rabbits, and thereby induced skin cancer experimentally for the first time. Since coal tar tends to dry up, I suppose that he succeeded in creating these skin cancers only by dissolving the coal tar in rape seed oil before application to the rabbits' ears.

At that time, unfortunately, this line of research was not further developed in Japan, and shifted to England. As is also true at the present time, even excellent research initiatives conceived in Japan are frequently not exploited in this country, thus, more than 50 years ago, this area of research shifted to the Royal Cancer Hospital in London and Department of Pathology at Oxford University, where exhaustive efforts were devoted to the elucidating the components of coal tar. The studies commenced with additional tests of same type, that is, coal tar was applied to the ears of rabbits or the backs of mice. Probably, a considerable quantity of rape seed oil was imported from Japan, but England being a European country, Italian olive oil and various other oils were also tested as media.

The journal "*Cancer Research*," published by the American Cancer Society, is the most authoritative journal relating to cancer, and was founded a 1941, just 50 years ago. No specialized journal relating to cancer existed in Britain at that time. If one peruses the first issue of this journal, one finds that reports on this type of research

appear throughout the entire issue, from beginning to end.

Professor Isaac Berenblum, a distinguished Israeli scientist, who is now retired in Tel Aviv, was then engaged in research at Oxford University. At present, he is well beyond 90 years of age, but at that time he must have been about 40. Subsequently, he worked for a long period at the Weizman Institute of Science in Israel. This institute is approximately 30 to 40 minutes by automobile from the city of Tel Aviv. When I was invited to the Institute's Annual Hams Lindner Lectures three or four years ago, Professor Berenblum came to the Institute from Tel Aviv especially for this occasion.

The experiments conducted by Professor Berenblum more than 50 years ago at Oxford University demonstrated that cancer is most readily induced by using coal tar together with the previously mentioned croton oil of Chinese tradition. The chemical structure of the relevant component of coal tar was established in 1930 by a researcher named Dr. Kenaway at the Royal Cancer Hospital in London. The application of this component alone does not readily induce cancer, but after once this component has been applied to the skin, if croton oil is subsequently applied several times, then conspicuous neoplasms develop. On the other hand, any number of applications of croton oil alone fail to induce cancers.

Therefore, one can conclude that carcinogenesis proceeds in two stages. As you all know, liver cirrhosis frequently progresses to hepatic carcinoma. Thus, the emergence of cancer requires initiation - probably what is now termed genetic damage – and "promotion," which induces the development of visible malignant lesions from this prior damage. This notion was clearly described by Professor Berenblum 50 years ago. In fact, this hypothesis is presented in his article on pp. 44-48 of the first issue of "*Cancer Research*" in 1941. In this context, a great deal of research in various cancer research institutes throughout the world was devoted to elucidating the identity of the "villain" lurking within the croton oil, and the specific mechanism of promotion of carcinogenesis, which until then had been merely a conceptual hypothesis.

The "villain" lurking in the croton oil was identified and its structure established thirty years ago by Dr. Hecker, now honorary director of the Heidelberg University Cancer Research Institute, and Dr. Van Duuren of New York State University. Figure 6 shows the chemical structure of the responsible constituent of croton oil. Rather complex moieties are present, but a structure resembling the aforementioned

“headless torso with one short leg” is attached to one portion of the molecule. The fact that this substance promotes carcinogenesis was established 30 years ago. Moreover, in addition to its cancer-promoting activity, the fact that, in the presence of calcium ions, this substance can reproduce the actions of various hormones was also documented.

I suddenly perceived the significance of these facts during the dead of night and was sleepless with excitement. Perhaps, I thought, this substance performs the functions of the “headless torso.” After examination from various viewpoints, this conjecture, which we had previously considered to he highly improbable, turned out to be correct! Although I shall not present the relevant experimental results during today’s lecture, the present substance manifests entirely the same type of activity as that of the “headless torso” at only one-thousandth of the required concentration of the latter. At the spring of the next year, I delivered a lecture at a conference specifically convened for specialists in that area at Squaw Valley in the United States, a skiing resort where, I understand, the winter Olympics have been held. Soon after the conclusion of my talk, corroborative and extended studies were conducted in the United States with respect to this line of research. As regards our own group, in view of what we had done up to that time, we felt compelled to devote our full efforts to the verification of the aforesaid hypothesis concerning the mechanism of hormonal action, irrespective of possible connections with cancer research.

As given in Figure 7, when hormones or other biologically active substances act upon a cell membrane, the hormone is sensed by an “antenna,” that is, a receptor, and the “headless torso” (diglyceride, DG) activates the proteinphosphorylating enzyme, that is protein kinase C (PKC). The “head” (IP_3) actually mobilizes calcium from its intracellular store, but at the time we still had not recognized this function of the head. Since the “headless torso” cannot enter the cell from the exterior, if instead one uses a “headless torso” with a short leg which does penetrate the membrane, or a tumor promoter, that is, the “villain” contained in croton oil, then the cell membrane will be penetrated, and the activity of protein kinase C (PKC) will be manifested. Therefore, the “antenna” portion will be completely bypassed, thus, if either of these agents is applied from outside the cell membrane, then the activity of protein kinase C the protein transphosphorylase (PKC) can be elicited without destroying the cell membrane.

On the other hand, since the importance of calcium ions had been realized long

before that time, there also arises the problem of clarifying this aspect of the phenomenon. Many pharmacologists are aware of agents which permit the permeation of calcium ions only, and if such an agent is allowed to act upon the cell membrane, then the intracellular calcium ion concentration can be raised. Such agents are known as calcium ionophores.

Therefore, by treating cells, not with natural biologically active substances such as hormones, but with an artificial membrane-permeating “headless torsos” or tumor promoters, and calcium ionophores, signal pathways for the two expected types of information transmission can be separately or simultaneously opened. The concept that cells can function by means of calcium ions was established in Britain over a period of more than a century. Thus, the question of whether this view is correct, or whether protein kinase C (PKC) is also necessary, or whether either one is sufficient, could be tested by simple experiments.

We proceeded to conduct such experiments. The experimental system consisted of platelets as a cell model, and, as a natural biologically active substance, thrombin or collagen, which acts upon the platelets and elicits secretion of serotonin and thereby causes platelet aggregation. Antigenic stimulation of mast cells causes the secretion of histamine. Leucocytes stimulated by foreign substances secrete an enzyme known as lysosome. Stimulation of lymphocytes by antigens elicits their proliferative response. The hypothesis under consideration could be experimentally verified with any of these cells as models.

On the basis of these various experimental facts, we advocated the hypothesis that both the calcium ions and the protein kinase C (PKC) pathways were indispensable for inducing cellular responses.

In December 1982, I was invited to visit the Royal Society of Britain, and presented a lecture on these research results. The Royal Society building stands arrayed with Buckingham Palace, close to Trafalgar Square. The entrance is small, but upon entering, one finds a truly magnificent structure. The Royal Society was founded during the 17th century, more than three centuries ago, and the second president of the society was Isaac Newton. Since that time, the society has kept a visitor’s book, wherein the original signatures of many distinguished scientists throughout modern history can still be found.

From the Japanese viewpoint, the European nations and Britain are remote antipodal countries, and from their viewpoint the same applies to Japan. However, although I failed to notice the fact at that time, many of the contemporary physiologists of Britain and the various European nations attended that conference. The one-way travelling time between London and Paris is 40 minutes, which is actually far more convenient and closer than a round trip from here to Tokyo. Therefore, a scientific community of this sort exists in the antipodal region of the globe, and this is an extremely important fact.

At that time, I was personally unacquainted with almost all of the European scientists arrayed there, who listened to my lecture with an apparent attitude of almost cold indifference but with keen vigilant eyes, as though pondering upon how they might utilize the content of my presentation in their own experimental systems. Hence, pursuant to my exposition of this topic, all the content would presumably be incorporated into the their own experimental systems, and thereafter my research group could not possibly emulate their rate of progress.

On that occasion, Professor Peter Baker, a professor at King's College and a scholar in the direct academic lineage of Professor Hodgkin and Huxley, was present in the audience. Unfortunately Peter passed away because of a heart attack some time ago. He subsequently mentioned to me when we had become well acquainted, that immediately after my talk, he returned to his laboratories and repeated the experiments in question. Thus, science progresses in this manner, through a series of instantaneous passing encounters!

This is merely one example, following this presentation, the concepts under consideration were promptly extended to researches subsuming a wide range of topics in medical sciences, including various processes involving secretory responses of nearly the entire endocrine and exocrine system, such as adrenalin secretion by the adrenal medulla, steroid hormone secretion by the adrenal cortex, insulin secretion, and pituitary hormone secretion, as well as secretion of digestive juice by the pancreas, and also release of neurotransmitters from nerve endings, and as I shall describe later on, many brain functions, memory, immune responses research on cancer, inflammation, the cardiovascular system, and so forth. Thus, a great many scientists commenced active research along these lines, and there finally arrived an epoch when this hypothesis was

rigorously tested. At that time, our group was entirely preoccupied with the so-called "headless torso." From my preceding remarks, I believe that you can well imagine the circumstances responsible for the fact that we were unable to devote our attention to the role of the severed "head."

Prior to my return to Japan from the Royal Society, I visited Cambridge University. Then, I met Dr. Mike Berridge, who is now one of my finest friends. During the month of December, the night at Cambridge is extremely long, with the sky already dark by 4 P.M. and the dawn not breaking until about 10 A.M. Incidentally, to digress for a moment, Cambridge is one of my favorite universities. Historically, the date of founding of this university is obscure, but, for example, there is a small physics laboratory at Cambridge, called the Cavendish Laboratory, and forty-eight scientists related to this laboratory alone have received the Nobel Prize. A series of major researches, ranging from the discovery of the atom to the discovery of isotopes, have been conducted at this laboratory, and this flourishing research activity is still continuing at the present time. Many instruments devised and hand fabricated by their originators, are still arrayed in the corridors of the building. Furthermore, original handwritten articles and notes by distinguished scholars still remain on many shelved cabinets. This is truly what could be called a scientific environment! Mike was born in Rhodesia, Africa, matriculated at Cambridge University, and for a long period of time conducted research on insect physiology, particularly the secretions of insect salivary glands. During the very year when I was studying at the Rockefeller University, he was studying overseas with Professor Ted Rall, a colleague of Professor Sutherland whom I mentioned earlier. Furthermore, during the year when I assumed a post at Kobe University, he returned to Cambridge University, and concurrently with studies on the aforementioned cyclic AMP, he also commenced researches in the British physiological tradition, linking calcium ions with phospholipids, considering, as I did, that phosphorus-32 was inexpensive and therefore could be appropriately utilized for this purpose. He utilized flies as experimental animals. The application of a certain hormone to the salivary glands induces a flow of saliva. He conducted his analysis using this experimental system, and stated that since he himself had not performed experiments using the so-called "headless torsos," he could not criticize my research. However, he believed that the "head" could not be ejected to no purpose but probably performs some

function. Remarking that I had been artificially introducing calcium ions from the exterior, he asked where I thought the calcium ions actually came from in vivo. This was indeed an incisive criticism; we had been unduly complacent in limiting our consideration to the “headless torso” I suppose that Mike, at that time had an idea that the “heads” were probably related to the action of calcium ions, but since three phosphate groups are also bound to each head, even if applied from the exterior, the head unfortunately could not enter the cell, and therefore the idea, although conceivable, could not be proved.

I thereupon returned directly to Japan, and things happened in the fall of the following year. I was surprised to find an account concerning the mere washing of cells with distilled water. This was a novel idea, but noting that this manipulation had previously been performed by a German lady physiologist, Dr. Schulz. Collaborating with her, Mike washed cells three or four times with distilled water. The cells membranes were thereby rendered highly permeable, without destroying the cells, thus the severed “heads” can enter from the exterior. Moreover, the calcium ion concentrations inside and outside the cell were equalized.

This experiment, was one in which cells that had been rendered permeable in this manner were placed in a beaker. Since the intracellular and extracellular calcium ion concentrations were equal, the calcium ion concentration could be accurately measured with a simple electrode. Furthermore, when ATP was added, since the cell membrane was permeable, the ATP also entered the cell. Thereupon, utilizing the energy provided by the ATP, the calcium ions were accumulated in the intracellular calcium store, thereby decreasing the ambient calcium ion concentration. After the calcium ion concentration had been thus decreased, the previously mentioned “heads” (IP_3) were added, and as a result the calcium ion concentration was again increased, albeit very slightly; that is, calcium ions were released from the intracellular store. The execution of this experiment required only five minutes, but this five-minute experiment was a product of his assiduous research efforts extending over a period of more than 20 years!

Such circumstances are particularly frequent in British scientific research. For example, even the aspirin experiment performed by Professor Vane was exasperatingly simple, but represented the culmination of a research history of two or three decades. Our group, focusing solely upon the activation of protein kinase C (PKC) by the

“headless torso,” had ignored the role of calcium ions, but the Cambridge University group had concluded that the “head” acts upon the intracellular calcium store and thereby induces the release of calcium ions.

Again, I shall confine myself to a very brief description. According to our conception, the arriving hormone acts upon the “antenna” (receptor), whereupon the action of an enzyme creates a decapitated torso, which elicits the activity of protein kinase C (PKC). Although we had merely been thrusting calcium ions into the cell from the exterior, Mike proposed the hypothesis that the calcium ions were, rather, produced by the action of the “head with the derby hat.” However, we finally understood the existence of a basic signal transduction pathway whereby various intracellular functions are controlled through coordinated action of both of these mechanisms (Figure 7).

Mike and his colleagues first announced this hypothesis at the research conference convened that autumn in Zeist, located in a forest on the outskirts of Utrecht in the Netherlands. However, at that time, the proposal of this hypothesis failed to evoke a particularly great response. Three months later, a conference of this type was also held at Dallas in the United States. After three months, a similar conference was held at Montreal in Canada; this time, the audience expressed praise for Mike. Thus, scientific trends are dynamic and can change rapidly within only a period of three months. Amidst these currents of change, I have scrutinized the trends in this field for more than a decade, and have concluded such circumstances are truly characteristic of science.

Through this course of events, the mechanisms of cellular signal transduction established through the combined efforts of Mike and his colleagues in Cambridge and our group were finally to be recognized by the scientific community.

As regards subsequent developments, I shall merely present an extremely brief and simple account of recent events in this area of research. Initially, we believed that protein kinase C (PKC) is a single homogeneous substance. Subsequently however, the introduction of so-called molecular biological techniques has revealed that PKC is actually a mixture of multiple enzyme protein molecules with closely related structures. As illustrated in Figure 8, this enzyme corresponds to an amplifier tube, so to speak. At present, structural variants of the enzyme are known, and in addition, many others with still unknown structures exist. The structures of these enzymes are all extremely similar, and their respective functions are also very similar, but the types of cells wherein they

occur and their intracellular localizations are all different, and their roles to amplify the extracellular signals are also different.

The category of biologically active substances includes many distinct varieties such as hormones, neurotransmitters, and cell growth factors, and therefore the types of signals delivered to cells are presumably quite varied. Hence, there is a high probability that the multiple enzymes with different roles are provided for cellular responses to these different types of extracellular signals. At present, researchers throughout the world are actively engaged in the analysis of these specific roles of the enzymes.

As one example, a certain type of the enzyme PKC is present only in the brain, particularly in the hippocampus, which plays a crucial role in memory functions. Many studies have been conducted throughout the world, including Japan, in connection with the functions of this type of enzyme in the brain, and in fact several academic conferences have been devoted to the relations of this enzyme with the release of neurotransmitters, regulation of neural conduction, memory functions and Alzheimer's disease, etc.

I have merely mentioned a single example, but as shown in Figure 9, the mechanism of hormonal action I have discussed today has been applied to researches in a wide range of biological and medical sciences, and in fact, has now apparently developed to the stage where applications are considered at various aspects on clinical medicine.

During the summer of the year before last, a conference was convened at Lake Placid in the United States in commemoration of the research results achieved by Mike and his group and by our group. Almost all of the researchers I have mentioned today assembled at that conference. The ejection of the "headless torso" was first reported in 1953 by Mabel and Low at Sheffield University in England. At that time, the significance of this phenomenon was not understood, but pursuant to this discovery in 1975, Bob in England conjectured that the ejected head was probably related to calcium ions. Then, pursuant to the announcement of this hypothesis, Mike at Cambridge University explained the function of the ejected "head." Our group in turn, pursuant to the researches in that connection which had been conducted in the United States, developed this line research further as well as learning from the relevant researches conducted in Europe, and thereby created, I believe, an extremely useful concept in this

field of research.

Japan is prone to pursue various currently fashionable trends. Fashionable trends are not necessarily undesirable: the crucial problem is that of progressing from such a trend to the next stage of development. Once a direction of research has been delineated, organization and economic power take control. However, that is industry, and not science. Industry or Engineering and science are distinct entities, and I believe that in contemporary Japan science and industry tend to be unduly confused with one another. World science is characterized by currents and traditions, and many researchers ponder upon their original ideas and devote their ingenuity to research problems for periods of several decades, considering the next stage of progress. This approach is, I believe, what Japan requires at the present time.

John Harvard graduated from Cambridge University and established a university at Boston some 360 years ago. Even the science of America, a nation certainly not handicapped by a lack of verbal expression, required two centuries in order to enter the international scientific community. A few years ago, Heidelberg University celebrated the 600th anniversary of its foundation. The University of Complutense in Spain will celebrate the 700th anniversary next year. The University of Paris, which boasts a history of some eight centuries, shifted to the Sorbonne a great many years ago; the liberal arts school, where Louis Pasteur and Victor Hugo served on the faculty, is now located there. The universities at Bologna and Padua in Italy are even older.

Several years ago, when I was invited to visit Padua University, I was guided through the premises by one of my friends, from whom I learned that human anatomy was first taught to students at the universities of Bologna and Padua in the year 1495. The cranial bones of the first professor of anatomy as well as his successors are still preserved there in a splendid box. The lecture theater used at that time is also still preserved. The students observed the demonstrations from above while standing on the steep conical slopes of the theater. In fact, Michelangelo was one of the students at the University of Padua during that period, and attended anatomy lectures as a medical student in that theater. Upon reflection, one can appreciate that sculpture of that quality could not have been created without a knowledge of anatomy. Furthermore, Copernicus was also a physician, and studied as a medical student at this university during the three-year period from 1500 to 1503.

Similarly, at the University of Uppsala in Sweden, the Department of Anatomy, a wooden structure, still remains intact. Another university anatomy department also existed during that period at the University of Leiden in the Netherlands. The anatomy departments of these three nations are the world's oldest. That period was contemporaneous with the Muromachi period in Japanese history. At that time, the professors taught science to their students at the risk of their lives. In the Orient, as you know, traditional Chinese medicine has existed since antiquity, but unfortunately, the lacked the spirit of experimental verification.

The University of Leiden in the Netherlands was founded slightly prior to Harvard University. Four years later, the University of Utrecht was established. Western academic knowledge in the medium of the Dutch language reached Japan during the year 1600, and the study of modern medicine in this country commenced with the treatise "*Kaitai Shinsho*." The 24th Japan General Conference of Medicine was convened at Kyoto in April last year, just one century after the first conference of the series held during the 23rd year of the Meiji period. Thus, Japanese medicine been in existence for just a mere century.

The issues to be confronted still lie before us. Since science does not suddenly display prodigious advances on any single day, the question is that of how Japan should proceed to participate more intimately in the international scientific community, which has evolved over a long period of many centuries. Perhaps still another century may be required in order to attain, the status of a genuinely full participant. Science is neither a type of amusement for dilettantes nor a sports competition, but a body of knowledge which continuous to expand, developing into a universal fund of human intellectual wealth. The seeds of scientific research are sown in the course of an endless series of human encounters, and these seeds are nurtured, develop into sturdy trees of knowledge, and finally bear precious fruit.

I'm afraid that my talk today has been rather desultory, and I'm not certain whether this sporadic account has succeeded in fully expressing my purport. Although I have deliberately avoided the description of specialized details, I should nevertheless be highly gratified if I have managed to convey some of my thoughts in this connection.

This has been a superb meeting here today, and I have been honored by an extremely courteous introduction. I am profoundly grateful for and hope that I have

done justice to this fine opportunity.

Thank you for your kind and patient attention.

図 1. 細胞間の情報伝達
Figure 1. Mode of Cell-to-Cell Communication

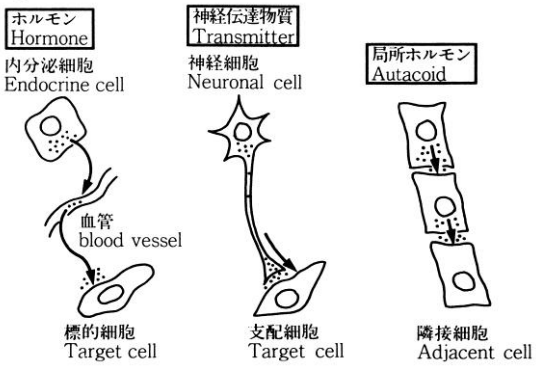


図 2. グリコーゲン・ホスホリラーゼ
Figure 2. Glycogen phosphorylase

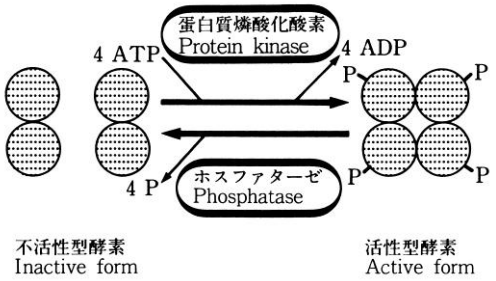
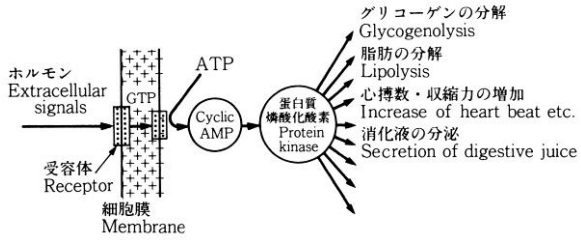


図 3. Cyclic AMPによる情報の受容と伝達
Figure 3. Signal Transduction through Cyclic AMP

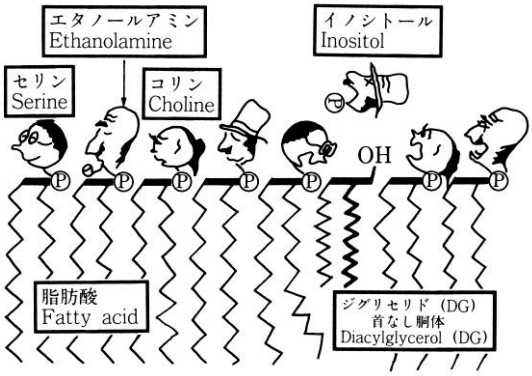


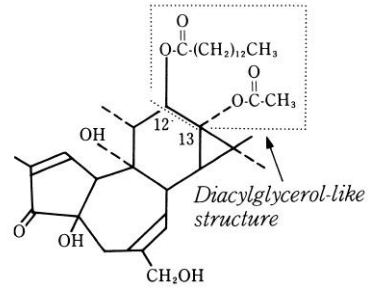
セカンドメッセンジャー学説 (Sutherland, 1958)
Second messenger theory (Sutherland, 1958)

図4. 生体の種々のホルモン・生理活性物質
Figure4. Various hormones and Physiologicaliy active substances

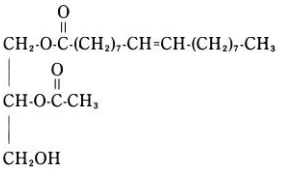
Cyclic AMPを介するホルモン Hormones acting through mediation of cyclic AMP	Cyclic AMPが関係しないホルモン Hormones independent of cyclic AMP	
アドレナリン(β作用) Adrenalin (beta action) ドーパミン(D1 作用) Dopamine (D1 action) ヒスタミン(H2 作用) Histamine (H2 action)	アセチルコリン(M作用) Acetylcholine (M action) ヒスタミン(D1作用) Histamine (D1 action) セロトニン(5HT2作用) Serotonin (5HT2 action)	ドーパミン(D2作用) Dopamine (D2 action) アドレナリン(α作用) Adrenalin (alpha action)
グルカゴン Glucagon 副腎皮質刺激ホルモン Adrenocorticotropic hormones 副甲状腺ホルモン Thyroid hormone セクレチン Secretin	アンギオテンシン Angiotensin バソプレシン Vasopressin コレシストキニン Cholecystokinin セルレイン Caerulein	ボンベシン Bombesin パンクレオザイミン Pancreozymin サブスタンスP Substance P ブラジキニン Bradykinins
プロスタグランジンE Prostaglandin E	トロンボキサン Thromboxanes トロンビン Thrombin コラーゲン Collagen	増殖促進因子 Growth promoting factors 化学遊走因子 Chematactic factors 分泌促進因子 Secretagogues

図5. 細胞膜イノシトールリン脂質の分解
Figure 5. Inositol Phospholipid Hydrolysis





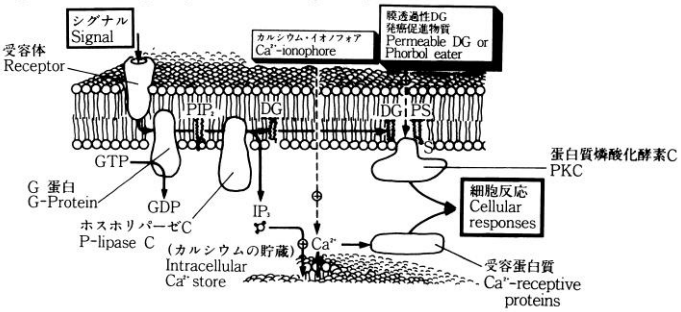
12-O-Tetradecanoylphorbol-13-acetate
発癌プロモーター
ホルボール・エステル(TPA)
Phorbol ester (TPA)



膜透過性
ジグリセリド (DG)
Permeable
Diacylglycerol (DG)

図6.
Figure 6.

図7. ホルモンの情報伝達系
Figure 7. PIP_2 Hydrolysis for Cell Signalling



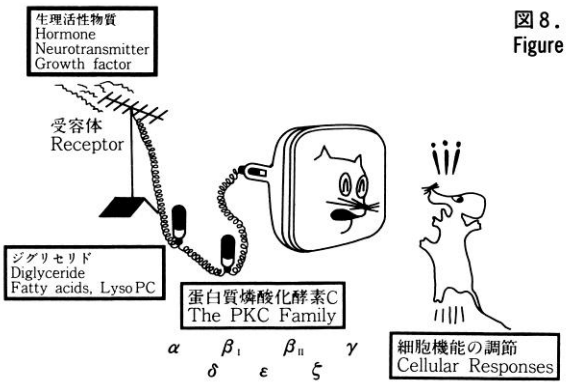


図8. 細胞内情報伝達の仕組み
Figure 8. Cell Signalling via PKC Family

図9．医学生理学研究への応用

内分泌研究	副腎髄質	アドレナリンの分泌
	副腎皮質	ステロイドホルモンの分泌
	膵ラ氏島	インシュリンの分泌
	脳下垂体	性腺刺戟ホルモンの分泌
		甲状腺刺戟ホルモンの分泌
		黄体化ホルモンの分泌
		乳腺刺戟ホルモンの分泌
		生長ホルモンの分泌
外分泌研究	膵臓	アミラーゼの分泌
	耳下腺	消化液の分泌
神経科学研究	末梢神経系	アセチルコリンの分泌
		神経刺戟伝播の促進
	中枢神経系	アセチルコン等の分泌
		シナプスの可塑性
免疫学研究	T－リンパ球	活性化の初期反応
	B－リンパ球	増殖分化の初期反応
癌研究	細胞一般	発癌の促進機構の解明
		各種の癌遺伝子の作用
炎症の研究	白血球	化学走性の発揮
		殺菌作用の発現
	肥満細胞	ヒスタミンの放出
	好塩基球	ヒスタミンの放出
	血小板	凝集及び活性化反応
		セロトニン放出
	線維芽細胞	増殖反応
心循環器研究	血管平滑筋	収縮・高血圧の発症
	心臓	収縮と弛緩

Figure.9 Proposed Roles of PKC in Cellular Regulation

Endocrine Systems	
Adrenal medulla	Adrenalin secretion
Adrenal cortex	Aldosterone secretion
Pancreatic islets	Insulin release
Pituitary gland	Gonadotropin release
〃	Tyrotropin release
〃	Growth hormone release
〃	Prolactin release
Hypothalamus	LH-RH release
Parathyroid gland	PTH release
Exocrine Systems	
Pancreatic acini	Amylase secretion
Parotid gland	Protein secretion
Gastric gland	Pepsinogen secretion
Submandidular gland	Mucin secretion
Nervous Systems	
Peripheral nerve endings	Acetylcholine release
Central nervous tissues	Transmitter release
〃	Channel regulation
〃	Neuronal plasticity
Inflammation & Immune Systems	
Platelets	Serotonin release
〃	Aggregation
Neutrophils	Superoxide generation
〃	Chemotaxis
Basophils	Histamine release
Mast cells	Histamine release
Lymphocytes	T-, B-Cell activation
Vasculoendothelial Systems	
Smooth muscle	Contruction
Endothel cells	Arachidonate release
Cell Proliferation & Differentiation	
Nearly all cell types	myc, fos gene activation
〃	Receptor induction