Prologue: Signal Transduction from an Historical Perspective

TRANSDUCTION, THE WORD AND ITS MEANING

The expression *signal transduction* first made its mark in the biological literature in the 1970s (Hildebrand, 1977) and appeared as a title word in 1979 (Springer et al., 1979; Koman et al., 1979; Kenny et al., 1979). Physical scientists and electronic engineers had earlier used the term to describe the conversion of energy, or information, from one form into another. For example, a microphone transduces sound waves into electrical signals. The term implies two related activities: one concerns transmission and the other translation of the original signal (a sound wave). Its widespread use in bio-speak was triggered by an important review by Martin Rodbell, published in 1980. He was the first to draw attention to the role of GTP and GTP-binding proteins in metabolic regulation and he deliberately borrowed the term transducer to describe their role in the relay of the receptor signal to the effector (Figure 1-1).

Alfred G Gilman and Martin Rodbell were awarded the Nobel Prize in 1994 “for their discovery of G-proteins and the role of these proteins in signal transduction in cells.”

In the year 2010, 12.6% of all papers using the term *cell* also employed the expression *signal transduction* and 16.6% also employed the expression *signaling* (the American spelling of *signalling*) (information from PubMed). The explosion in signal transduction research corresponds with the episode in which it became apparent that oncogenes disrupt ordinary, well-controlled, signaling processes. In particular Ras, the product of the oncogene *ras* leading to the formation of rat sarcoma, and its role in growth factor signaling has been the subject of intense investigation.
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FIGURE 1-1 The transducer. Introduction of the concept of a transducer in the relay of the receptor signal to the effector. A transducing GTP-binding protein relays the receptor signal, we speak of signal transduction. Adapted from Rodbell (1980). Image of Rodbell from http://www.nobelprize.org/.

FIGURE 1-2 Occurrence of the term signal transduction. The left-hand axis records all papers using the term cell traced through the PubMed database. The right-hand axis records the proportion of papers using the term cell that also use the term signal transduction. Major discoveries that have boosted signal transduction research are shown underneath the time axis.

It occurs that signaling mechanisms are an important research domain in biological sciences (Figure 1-2).

Below follows a description of personalities and experiments, during the transition from the nineteenth to the twentieth century, that have paved the way to our current understanding of how different parts of the body communicate with one another (hormones, neurotransmitters,
growth factors). Naturally, major discoveries gave rise to controversy (as they challenged hegemony of ideas and personalities) and, as a consequence, a good dose of (persistent) anxiety for those who deviated from the trodden path. Our account also shows that in many instances, scientists did not really know what to look for and thus what to expect from their experiments. Only through the course of their experimentations did they develop a sense of direction and importantly, understanding. Had they been confronted with current grant application forms, the section “expected outcome” would be manifested by its blankness.

Transduction entries in the Oxford English Dictionary (Figures 1-3 and 1-4)

IRRITABILITY, A VITAL PHENOMENON

The origin of life is often considered as fundamentally a problem of the origin of template replication. However, replication itself is not
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transduction

(trans'dak'ʃən, trens-) [ad. L. transduction-em (usually traductionem), n. of action f. tra (ns)dācre: see TRADUCE.]

1. The action of leading or bringing across. rare.

1656 BLount Glossog. Transduction, a leading over, a removing from one place to another. a1816 BenthAm Offic. Apt. Maximized, IntroD. View (1830) 19 In lieu of adduction, as the purpose requires, will be subjoined abduction, transduction, and so forth.

2. The action or process of transducing a signal.

1947 Jrnl. Acoustical Soc. Amer. xix. 307/1 It is rather interesting that the indirect method of electronic transduction, instead of the indirect method of employing a conventional transducer and then amplifying the output with a vacuum tube, has not been developed. 1970 J. EARL Tuners & Amplifiers iv. 87 Low impedance pickup cartridges. using the moving-coil principle of transduction. 1975 Nature 17 Apr. 625/1 The transduction of light energy into neural signals is mediated in all known visual systems by a common type of visual pigment.

3. Microbiology. The transfer of genetic material from one cell to another by a virus or virus-like particle.

1952 Zinder & Lederberg in Jrnl. Bacteriol. LXIV. 681 To help the further exposition of our experiments, we shall use the term transduction for genetically unilateral transfer in contrast to the union of equivalent elements in fertilization. 1960 [see F III. 1]. 1971 Nature 18 June 466/1 It has been suggested that transduction of genes by viruses was an important mechanism in evolution for spreading useful mutations between organisms not formally related. 1977 Lancet 9 July 94/2 These were derived by selection of sensitive variants from gentamicin-resistant strains or by transduction of this resistance to sensitive strains.

Hence transductional a., of or pertaining to (genetic) transduction.

1956 Genetics xli. 845 (heading) Linear inheritance in transductional clones. 1980 Jrnl. Gen. Microbiol. cxix. 51 Transductional analysis revealed that one of the four mutations carried by strain T-693 was responsible for constitutive synthesis of both isoleucine and threonine biosynthetic enzymes.


sufficient, metabolism was another important property right from the beginning (Dyson, 1999). According to Thomas Henry Huxley (Figure 1-5), a third essential element for the living comprises a stimulus-response system. Ultimately, this system became the basis of how organisms respond to the environment, and how parts of an organism (or whole organisms) communicate with one another. In his lecture on
“the physical basis of life” (Edinburgh, on the evening of Sunday November 8, 1868), Huxley argues that all living things, ranging from Amoebae to Homo sapiens, are substantially similar in kind with respect to elementary functions and substance (which he named “protoplasm”). Irritability, Huxley’s description of stimulus-response coupling, takes an essential place among the list of “vital phenomena.”

In physiological language this means that all the multifarious and complicated activities of man are comprehensible under three categories: either they are immediately directed towards the maintenance and development of the body (“metabolism”), or they effect transitory changes in the relative positions of parts of the body (“stimulus-response”), or they tend towards the continuance of the species (“template replication”). Even those manifestations of intellect, of feeling, and of will, which we rightly name the higher faculties, are not excluded from this classification, inasmuch as to everyone but the subject of them, they are known only as transitory changes in the relative positions of parts of the body. Speech, gesture, and every other form of human action are, in the long run, resolvable into muscular contraction, and muscular contraction is but a transitory change in the relative positions of the parts of a muscle. But the scheme which is large enough to embrace the activities of the highest form of life, covers all those of the lower creatures. The lowest plant, or animalcule, feeds, grows, and reproduces its kind. In addition, all animals manifest those transitory changes of form which we class under irritability and contractility; and, it is more than probable, that when the vegetable world is thoroughly explored, we shall find all plants in possession of the same powers, at one time or other of their existence.
IRRITABILITY

Perhaps it were the medusa that he studied during his voyage on the HMS Rattlesnake (around 1846), that led Huxley to employ the word “irritability” and “contractility” (rather than stimulus-response coupling). Describing the stomach, situated under the disc of Rhizostoma he writes: “From this ‘common canal’ a series of parallel diverticula are given off at regular intervals, and run to the edge of the branch, where they terminate by rounded oblique openings. It is not always easy to see these apertures, but I have repeatedly satisfied myself of their presence by passing a needle or other delicate body into them. The difficulty in seeing the openings arises in great measure from the presence of a membrane which surrounds and overlaps them, and being very irritable, contracts over them on being touched” (Huxley, 1849).

CELLULAR THEORY AND PROTOPLASM

The initial microscopic observations of cellular structures by Robert Hooke (around 1653) and Anthony van Leeuwenhoek (around 1682) obtained full recognition when in 1832 the German botanist Matthias Schleiden proclaimed that cells were the elementary structures of plants. A few years later, Theodore Schwann showed that the animal tissues were also made up of cells, and that they owed their beginning and development to the activity of cell elements; thus originated the “cellular theory.” Until 1888, the composition of the brain remained, however, an enigma. In that year Santiago Ramon y Cajal, using Camillo Golgi’s silver nitrate impregnation staining, convincingly demonstrated that even the soft, near homogeneous, gray matter was made up of distinctive structures, the nerve cells.

In his “Manual of Physiology” (1889) Gerald Yeo, from King’s College, describes the characteristics of the cell as follows: “The first idea which was conveyed by the term cell varied much from that which we now accept as a proper definition of such an organic unit. Fully developed vegetable cells being the first discovered were taken as the type of all. The main characteristics of these may be briefly summed up. First, a membranous sac called the cell wall, generally very well defined, and, secondly, within the cell wall various cell contents. Among the more conspicuous of the latter may be mentioned (1) a soft, clear, jelly-like substance called protoplasm, in which lies a nucleus, and (2) certain cavities called vacuoles, which are filled with a clear fluid or cell sap” (Yeo, 1889).
At that time, the English scientific establishment was still closely tied to the Church of England, while science was part of “natural theology.” Scientists merely played the role of explaining the book of Genesis in biological terms. Both Darwin’s findings about the origin of species (On the origin of species, published in 1859) and Huxley’s findings of common “protoplasm” strongly conflicted with the beliefs that species were unchanging parts of a designed hierarchy and that humans were unique, unrelated to other animals (or any species). Huxley, therefore, in a humorous way, cautions his audience about possible conflict with religious beliefs: “But I bid you beware that, in accepting these conclusions, you are placing your feet on the first rung of a ladder which, in most people’s estimation, is the reverse of Jacob’s, and leads to the antipodes of heaven. It may seem a small thing to admit that the dull vital actions of a fungus, or a foraminifer (protozoa), are the properties of their protoplasm, and are the direct results of the nature of the matter of which they are composed. But if, as I have endeavoured to prove to you, their protoplasm is essentially identical with, and most readily converted into, that of any animal, I can discover no logical halting-place between the admission that such is the case, and the further concession that all vital action may, with equal propriety, be said to be the result of the molecular forces of the protoplasm which displays it.”

Quite astonishingly, he ends the lecture as a molecular scientist avant la lettre, by interpreting the “transitory changes in the relative positions of parts of the body” as molecular changes! He writes: “And if so, it must be true, in the same sense and to the same extent, that the thoughts to which I am now giving utterance, and your thoughts regarding them, are the expression of molecular changes in that matter of life which is the source of our other vital phenomena” (Lecture source at: http://human-nature.com/darwin/huxley/chap6.html).

And indeed, we now know that as you have read these lines, thousands of GDPs have been replaced by GTPs, rapidly followed by their hydrolysis, thousands of phosphates have been passed from ATPs to proteins and back again (but indirectly), stretches of DNA have been methylated and certain histones have been acetylated. All this activity in the hope that you have not only provided meaning to these words, but also will remember some of them!

**MOLECULE**

The word molecule is derived from *molecula*, diminutive of the Latin word *mole* and, therefore, translated as “small mass.” Although the term was used widely at the onset of the nineteenth century, it was not until 1873, well after Huxley’s lecture, that, the Scottish physicist James Clerk Maxwell published an article in *Nature* in which he defines the term molecule as we still handle it today “An atom is a body which cannot be cut in two; a **molecule** is the smallest possible portion of a particular substance.”
FLESH AND GRASS, GERRIT MULDER AND JOHANNES BRAHMS

That the composition of plant and man is essentially not that different was already revealed by the Dutch chemist/physician Gerrit Mulder in 1835, working in Rotterdam, when he showed that egg white is composed of a base (“wortelstof”) comprising carbon, hydrogen, and nitrogen to which is added a pinch of phosphor and sulfur. This base (essentially an amino acid) was present both in animals and plants. According to his own words “I am the first to show that the flesh is present in the bread and the cheese in the grass” (in old Dutch: “dat het vleesch in het brood aanwezig is en de kaas in het gras”). On advice of Jöns Jacob Berzelius (Stockholm, Sweden), he named the omnipresent base-substance “protein.” Derived from the Greek proteios (or protos), meaning “first” (in this context, “most abundant” or “most eminent”).

In the same year as Huxley lectures about the physical basis of life, Brahms finishes his “Ein Deutsches Requiem” with a second movement entitled “For all flesh is as grass” (in German, “Denn alles Fleisch, es ist wie gras”). Here, however, the oratorio wishes not to reveal the similarity between man and plants in a chemical sense, quite the opposite, it wishes to accentuate the supremacy of the Divine by quoting Peter: “man is like grass, and like grass its glory withers and its flowers fall away, but the Lord endures forever.”

About 140 years later, in his book Creation: life and how to make it, Steve Grand, the father of the android robot Lucy and of the tribe of computer creatures called Norns, goes as far as to declare that life “is made not of atoms, it is merely built out of them. What life is actually ‘made of’ is cycles of cause and effect, loops of casual flow. These phenomena are as real as atoms – perhaps even more real” (Grand, 2001). To stress that physical matter is not essential for our lives he gives the example of long-range memories: “by now I hope you have thought of an experience from your childhood. Something you remember clearly, something you can see, feel, maybe even smell, as if you were really there. After all, you really where there at the time, weren’t you? How else would you remember it? But here is the bombshell: you weren’t there. Not a single atom that is in your body today was there when that event took place. Every bit of you has been replaced many times over (which is why you eat of course).”

Matter flows from place to place and momentarily comes together to be you. Whatever you are, therefore, you are not the stuff of which you are made. To see yourself as persistent phenomenon, when the substrate from which you are made is in constant flux, is to begin to understand life, and more than just life.
If this is all too esoteric, the example of the Golgi apparatus may perhaps be revealing to explain how “persistence” and “flux” relate to each other. As you probably will have seen or even studied in numerous electron micrographs, the Golgi comprises stacks of four to eight membrane-enclosed discs, the so-called cisternae, surrounded by numerous small transport vesicles. That is the static picture. However, if you block the transition of ERGIC to the cis-Golgi cisternae (by brefeldin A or dominant negative Arf1), a near-immediate dissolution of the organelle ensues and it only takes 5 min to eliminate the entire Golgi from the cell (Figure 1-6) (Ward et al., 2001). The electron-micrograph stills are deceiving because the Golgi is not a stable but a steady-state organelle: it exists as a consequence of a continuous flow of vesicles, trafficking both in an antro- and retrograde fashion (up and down), undergoing fusion (giving rise to cisternae) and fission.

In this book, we write abundantly about flux, of how cells constantly create new networks through subtle changes in protein shapes and protein composition (posttranslational modifications), leading to new protein interactions, altered subcellular localization or altered...
activity or any combination of these, and forming the basis of cellular persistence.

Changes in the wiring of proteins, this time through addition of new protein-interaction domains at the genomic level, also underlie the evolutionary process; coupling stimuli to different (new) responses. Analysis of whole genomes from different organisms revealed two important facts in this respect. First, when the genome (protein-coding genes) of the organism increases in size, the proportion of genes dedicated to cellular signaling increases as well (Figure 1-7(a)), from roughly 20–30%. Second, more detailed analysis of the domain architecture of the proteins involved in signaling reveals that, on average, animals have much more complex domain architectures than plants or protists (Figure 1-7(b)). What this means is that animals not only have bigger genomes, with many more signaling proteins, these proteins also can branch to a larger number of other signaling components. Together this leads to significantly denser signaling networks. Lastly, comparison between genomes of protists points to a greater variety in regulatory proteins than expected from the relatedness of the lineages (Anantharaman et al., 2007). This is taken to mean that signaling proteins determine eukaryotic diversity, that is, differences in signaling networks, connecting receptors with gene transcription, signify differences in species; again, signaling matters.

FIGURE 1-7 Signaling matters (a) Nonlinear scaling of the total number of signaling proteins in eukaryotes plotted against the number of protein-coding genes (representing the proteome). (b) Complexity quotient plot for signaling proteins. The complexity quotient for an organism is defined as the product of two values: the number of different types of domains that co-occurs in signaling proteins and the average number of domains detected in these proteins. The complexity quotient is plotted against the total number of signaling proteins in given organisms. Ata, *Arabidopsis thaliana* (plantae); Ddis, *Dictyostelium discoideum* (amoeboza); Tthe, *Tetrahymena thermophila* (protozoa). Image adapted from Anantharaman et al. (2007).
In his book *Wetware*, Denis Bray, takes the point that cells are computing devices, clever cells employing algorithms with Boolean logic (AND, NOT, OR), able to store information, memorize current or even past conditions, and being able to take decisions in a calculated manner (Bray, 2009). Cells calculate not through electric networks but, as mentioned above, through molecular networks, implying all major cellular components (lipids, sugars, proteins, and nucleotides). Each of these components acts as a switch as they exist in different shapes (allostery) or compositions or both. Each component may thus act as a binary switch but collectively they seem to give rise to analogous responses. Binary sets that do not act as two-valued logic (on/off) are said to follow the path of fuzzy logic (with truth values ranging in degree between zero and one) but here we start to maneuver on very thin ice. The book rightly makes the point that currently we are not able to translate the innumerable cellular processes into a comprehensive system (or comprehensive systems), which would allow us to predict or calculate cellular responses in silico. There are still too many unknown variables (like missing proteins, unknown quantities and enzymatic rates, undiscovered catalytic and allosteric functions) to create a comprehensive signaling system for animal cells. In modern jargon, the mammalian interactome is far from complete. Moreover, to return to Steve Grand, we describe signaling processes (fluxes) as ordered linear events, with hierarchal order—starting at the membrane and ending in the nucleus—but you should be aware that they are not. All processes described are parts of a web where the prime cause of any particular circumstance is under the influence of the effect. Glands secrete hormones (cause) that affect cellular activity (effect), and altered cellular activity (cause) in turn affects secretion of the hormone by

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**PROTISTS**

They are a diverse group of eukaryotic microorganisms. Historically, protists were treated as the kingdom Protista, which includes mostly unicellular organisms that do not fit into the other kingdoms, but this group is contested in modern taxonomy. Instead, it is better regarded as a loose grouping of 30 or 40 disparate phyla with diverse combinations of trophic modes (they rely on organic nutrition), mechanisms of motility, cell coverings, and life cycles. The protists do not have much in common besides a relatively simple organization—either they are unicellular or they are multicellular but without specialized tissues. This simple cellular organization distinguishes the protists from other eukaryotes, such as fungi, plants, and animals. Protists have two subkingdoms: algae and protozoa.

*Source: Wikipedia*
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the gland (effect), etc. In the words of Steve Grand “every cause is the effect of at least one other cause” and “control is therefore as much an effect as a cause.” Despite its immense potential in helping to understand cellular functioning, we will only briefly deal with the subject of “systems biology” because we feel that it does not yet facilitate the understanding for those who enter the field of signal transduction.

PROTOENDOCRINOLOGISTS

Despite excellent anatomical descriptions, almost nothing was known of the functions of the various organs, which constitute the endocrine system (glands) until the last decade of the nineteenth century. Indeed, in the standard textbook of the period (Foster’s Textbook of Physiology, 3 volumes and more than 1200 pages), consideration of the thyroid, the pituitary, the adrenals (“suprarenal bodies”), and the thymus is confined to a brief chapter of less than 10 pages, having the title “On some structures and processes of an obscure nature.”

In writing these paragraphs, we have relied heavily on the work of Victor Medvei (1993), Michael Aminoff (1993), and Horace Davenport (1991).

The initial impetus prompting the systematic investigations that led to the discovery of the hormones can be ascribed to a series of papers that were much misunderstood (Medvei, 1993; Aminoff, 1993; Davenport, 1991). However, here we are confronted with the work of Charles-Edouard Brown-Séquard, the successor of Claude Bernard at the Collège de France and a member of leading scientific academies also in England and the USA. He had held professorial appointments at both Harvard and Virginia; in London he was appointed physician at the National Hospital for the Paralysed and Epileptic (now the National Hospital for Neurology and Neurosurgery). He was an associate of Charles Darwin and Thomas Huxley. He wrote over 500 papers relating to many diverse fields such as the physiology of the nervous system, the heart, blood, muscles and skin, the mechanism of vision, and much more. He was an outstanding experimentalist making fundamental contributions. Starting with his doctoral thesis, he described the course of motor and sensory fibers in the spinal cord, a field to which he returned many times. He was in constant demand as lecturer, teacher, and physician on both sides of the Atlantic, crossing the ocean on more than 60 occasions. Of direct relevance to us must be his demonstration that the adrenal glands are essential to life.

In view of all this, it is curious that Brown-Séquard is now all but forgotten (Figure 1-8). On the rare occasions when he is recalled, it is generally
in connection with a series of brief reports, published in 1889, in which he described the self-administration by injection, of testicular extracts, which he considered had the effect of reinforcing his bodily functions.

Some brief quotations from his paper in the Lancet must suffice (Brown-Séquard, 1889a).

I am seventy-two years old. My general strength, which has been considerable, has notably and gradually diminished during the last ten or twelve years. Before May 15th last, I was so weak that I was always compelled to sit down after about half an hour’s work in the laboratory. Even when I remained seated all the time I used to come out of it quite exhausted after three or four hours of experimental labour…

The day after the first subcutaneous injection, and still more after the two succeeding ones, a radical change took place in me, and I had ample reason to say and to write that I had regained at least all the strength I possessed a good many years ago…

My limbs, tested with a dynamometer for a week before my trial and during the month following the first injection, showed a decided gain of strength…

I have measured comparatively, before and after the first injection, the jet of urine in similar circumstances - i.e., after a meal in which I had taken food and drink of the same kind and in similar quantity. The average length of the jet during the ten days that preceded the first injection was inferior by at least one quarter of what it came to be during the twenty following days. It is therefore quite evident that the power of the spinal cord over the bladder was considerably increased…

I will simply say that after the first ten days of my experiments I have had a greater improvement with regard to the expulsion of faecal matters than in any other function. In fact a radical change took place, and even on days of great constipation the power I long ago possessed had returned…

Finally, in a footnote:

It may be well to add that there are good reasons to think that subcutaneous injections of a fluid obtained by crushing ovaries just extracted from young or adult animals and mixed with a certain amount of water, would act on older women in a manner analogous to that of the solution extracted from the testicles injected into old men.
Possibly Brown-Séquard should be regarded as the father of hormone replacement therapy (HRT) (Spiegel, 1996), but he was certainly not the inventor of organotherapy, the attempt to cure human disease by the introduction of glandular extracts from other animals. Indeed, Gaius Plinius Secundus (known as Pliny the Elder, 23–79 CE) recorded his contempt of the Greeks who used human organs therapeutically, although he did recommend the use of animal tissues, in particular, that the testicles of animals should be eaten in order to cure impotence or to improve sexual function in men, and that the genitalia of a female hare should be eaten by women in order to achieve pregnancy (Medvei, 1993).

Fritz Spiegel (1996) reminds us that in Chinese medicine the effect of HRT is allegedly achieved by the administration of rhinoceros horn

Ever mindful of the possibility of autosuggestion, Brown-Séquard pleaded that others should examine his claims and to consider them with
objectivity. What a hope! But if any doubts remained, he went on record saying (Brown-Séquard, 1889b):

Not only is there no occasion for surprise that the introduction into the blood of principles derived from the testicles of young animals should be followed by an increase in vigour: such a result should indeed have been foretold. For in point of fact all the evidence shows that in the adult or elderly male the power of the spinal cord and also, albeit to a lesser degree, that of the brain is subject to variations linked to the functional activity of the testicles. In addition to the facts mentioned in this regard at the meeting of June 1st, I believe I should add that the following particularities have been observed on many occasions during the course of several years in the case of two individuals aged between 45 and 50… On my advice, each time they had to undertake a great physical or intellectual task they put themselves into a state of sexual excitement, while nevertheless avoiding ejaculation. The testicles became substantially active for a time and this was soon followed by the desired increase in the power of the nervous system.


And respectable it remained, anyway in France, where in the 1920s Serge Voronoff was treating elderly gentlemen by implanting sections of chimpanzee or baboon testicles, the so-called “monkey glands” (Borrell, 1976). R V Short (Murray, 1891) speculates that his patients may have been the first humans to become infected by HIV-1. Were it not for the fact that they remained impotent, the disease might have exploded on a world even less prepared than it was at the century’s end. One might reasonably imagine that an AIDS epidemic originating at that time could have changed the course of twentieth century history.

News travels fast and news of this sort travels faster still. Within weeks this was the subject of an editorial paragraph in the British Medical Journal under the heading The Pentacle of Rejuvenescence (Editorial annotation, 1889).

Many influential members of the English scientific establishment, however, strongly renounced his experiments, condemning them as “disgusting” and acts of “self abuse.” Despite this, like it or not, many physicians, aware of Brown-Séquard’s publications, were eager to test the possibilities of applying various organ extracts in their practice. Within a year or two, organotherapy was becoming respectable (Editorial annotation, 1925; Short, 1999). In a letter to his colleague Jacques Arsène d’Arsonval (quoted by Borrell (1976)), Brown-Séquard wrote that “the thing” was “in the air.” What made it especially so
may have been George Redmayne Murray’s report (Murray, 1891) on the treatment of myxedema. He described a patient who was treated with regular injections of sheep thyroid extract and went on to survive for further 28 years.

Henry Hallett Dale’s description of the discovery of an active substance extracted from adrenal glands (adrenaline) cannot be bettered (Dale, 1948; Schäfer, 1908):

Dr George Oliver, a physician of Harrogate, employed his winter leisure in experiments on his family, using apparatus of his own devising for clinical measurements. In one such experiment he was applying an instrument for measuring the thickness of the radial artery; and, having given his son, who deserves a special memorial, an injection of an extract of the suprarenal gland, prepared from material supplied by the local butcher, Oliver thought that he detected a contraction, or, according to some who have transmitted the story, an expansion of the radial artery. Which ever it was, he went up to London to tell Professor Schäfer what he thought he had observed, and found him engaged in an experiment in which the blood pressure of a dog was being recorded; found him, not unnaturally, incredulous about Oliver’s story and very impatient at the interruption. But Oliver was in no hurry, and urged only that a dose of his suprarenal extract should be injected into a vein when Schäfer’s own experiment was finished. And so, just to convince Oliver that it was all nonsense, Schäfer gave the injection, and then stood amazed to see the mercury mounting in the arterial manometer till the recording float was lifted almost out of the distal limb.

Within a few months, Oliver and Schäfer had demonstrated that the primary effect of the extract is a profound arteriolar constriction with a resulting increase in the peripheral resistance (Oliver and Schäfer, 1894, 1895). Their colleague Benjamin Moore reported that the activity could be transferred by dialysis through membranes of parchment paper, that it is

Schäfer’s own account of his first meeting with George Oliver relates events that may have taken place on an another planet from Dale’s version (Schäfer, 1908):

In the autumn of 1893 there called upon me in my laboratory in University College a gentleman who was personally unknown to me, but with whom I had a common bond of interest – seeing that we had both been pupils of Sharpey, whose chair at that time I had the honour to occupy. I found that my visitor was Dr George Oliver, already distinguished not only as a specialist in his particular branch of medical practice, but also for his clinical applications of physiological methods. Dr Oliver was desirous of discussing with me the results which he had been obtaining from the exhibition by mouth of extracts of certain tissues, and the effects which these had in his hands produced upon the blood vessels of man, as investigated by two instruments which he had devised – one of them, the haemodynamometer, intended to read variations in blood pressure, and the other, the arteriometer, for measuring with exactness the lumen of the radial or any other superficial artery. Dr Oliver had ascertained, or believed that he had ascertained, by the use of these instruments, that glycerin extracts of some organs produce diminution in calibre of the arteries, and increase of pulse tension, of others the reverse effect.
insoluble in organic solvents but readily soluble in water, resistant to acids and to boiling, etc. (Moore, 1895).

**HORMONES AND NEUROTRANSMITTERS**

These chemical messengers...or “hormones” (from ὁρμάω, meaning I excite or I arouse), as we may call them, have to be carried from the organ where they are produced to the organ which they affect, by means of the bloodstream, and the continually recurring physiological needs of the organism must determine their repeated production and circulation throughout the body. Ernest Henry Starling (1905)

Hormones are blood-borne “first messengers,” usually secreted by one organ (or group of cells) in response to an environmental demand to signal a specific response from another. The first such messenger to be endowed with the title of hormone was secretin, later shown to be a peptide released into the bloodstream from cells in the stomach lining, indicating the presence of food and alerting the pancreas. In the words of William Maddock Bayliss (codiscoverer with Ernest Henry Starling),

There are a large number of substances, acting powerfully in minute amount, which are of great importance in physiological processes. One class of these consists of the hormones which are produced in a particular organ, pass into the blood current and produce effects in distant organs. They provide, therefore, for a chemical co-ordination of the activities of the organism, working side by side with that through the nervous system. The internal secretions, formed by ductless glands, as well as by other tissues, belong to the class of hormones. Bayliss (1924)

In fact, adrenaline, the signal for fright-fight-or-flight, is a much better candidate for the accolade of “first hormone” (Cannon, 1929). Together with noradrenaline it is secreted into the bloodstream in consequence of emotional shock, physical exercise, cold, or when the blood sugar concentration falls below the point tolerated by nerve cells. Extracts having the activity of adrenaline, enhancing the force and volume of the heart output had been reported 10 years before the discovery of secretin, almost

**ADRENALINE VERSUS EPINEPHRINE**

It has been customary in Europe to give the substance 4-[1-hydroxy-2-(methylamino)ethyl]-1,2-benzenediol, alternatively 3,4-dihydroxy-α-[(methylamino)methyl]benzyl alcohol the name adrenaline. In the USA, the same substance is called epinephrine. Why have the Europeans employed the Latin (ad ren(es)) roots while the Americans go for the Greek (epi nephr(os))? John Jacob Abel, America’s first professor of Pharmacology, is credited with the isolation of the first hormone from adrenal glands as a pure crystalline compound but he failed to determine its composition correctly.
1. SIGNAL TRANSDUCTION FROM AN HISTORICAL PERSPECTIVE

ADRENALINE VERSUS EPINEPHRINE (cont’d)

This is credited to Jokichi Takamine, a chemist who had an arrangement with the Parke, Davis Company (Detroit, Michigan), who provided the formula C₉H₁₃NO₃. The company lost little time in protecting (by a patent) and marketing the preparation under the trademark of “adrenalin” (no terminal “e”), thereby, preventing the use of the same name by the American scientific community. Abel gave the alternative name epinephrin (again, no terminal “e”) to his compound. Independent reports from Dakin and Stolz of complete chemical syntheses of the racemic mixture of the two optically active isomers came in 1904 and 1905 (Stolz, 1904; Dakin, 1905). The European Pharmacopoeia now also indicates the use of the term epinephrine.

We justify our preference for adrenaline not only on its historical priority but also for reasons of logic and common usage. Who ever heard of epinephric receptors? Who ever used the expression “that really gets my epinephrine up”?

simultaneously, by George Oliver and Edward Schäfer in London (Oliver and Schäfer, 1894, 1895), and by Napoleon Cybulski and Szymonowicz in Krakow (Bilski and Kaulbersz, 1987).

In comparison with the ready acceptance of the principle of blood-borne transmission of chemical signals between organs, the idea of chemical (neurotransmitter-operated), as opposed to electrical transmission of impulses between nerves and nerves, and between nerves and muscles had a long gestation. The phenomenon of vagal stimulation causing a slowing of the heart, had been described in 1845 by Weber (see Bacq’s account in 1975) and the possibility of chemical transmission of this signal was proposed as early as 1877 by Dubois-Reymond:

Of known natural processes that might pass on excitation, only two are, in my opinion, worth talking about: either there exists at the boundary of the contractile substance a stimulatory secretion in the form of a thin layer of ammonia, lactic acid, or some other powerful stimulatory substance; or the phenomenon is electrical in nature.

It took all of 60 years for the principle of chemical transmission to achieve acceptance as the general means of communication between nerves and muscles. Otto Loewi recorded (Loewi, 1960) that he had conceived the idea of chemical transmission between nerves as early as 1903, but that at that time (Davenport, 1991).

I did not see a way to prove the correctness of this hunch, and it entirely slipped my memory until it emerged again in 1920… The night before Easter Sunday of 1920,
I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then I fell asleep again. It occurred to me at six o’clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at three o’clock, the idea returned. It was the design for an experiment to determine whether or not the hypothesis of a chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design.

**Henry Hallett Dale** and **Otto Loewi** shared the Nobel Prize in 1936 for “their discoveries relating to chemical transmission of nerve impulses.”

Henry Dale also contributed to the discovery of oxytocic action of pituitary extracts and he greatly contributed to the understanding of histamine-induced anaphylaxis and on the general conditions of shock.

Loewi relates how, in his experiment, he induced contractions by electrical stimulation of the vagal nerve in an isolated heart. He then transferred some of the fluid from this heart into the ventricle of a second heart undergoing similar stimulation. The result was to slow it down and reduce the force of contraction. He gave the name “vagusstoff” to the inhibitory substance, later identified as acetylcholine.

One might have thought that this demonstration of chemical, not electrical communication between hearts should have settled the issue. However, it is doubtful whether the technique that he used could have delivered the results that he described. There were problems of reproducibility, which may relate both to the temperature of the laboratory and to the seasonal variations in the response of the amphibian heart. In the winter months, the inhibitory fibers predominate so that electrical stimulation suppresses the rate and force of contraction. In the summer, the situation is reversed. Other problems arise from the transient nature of the pulse of the neurotransmitter. Eventually, with the efforts of many others, these difficulties were overcome. Even so, in order to prove the role of acetylcholine in neurotransmission it remained necessary to demonstrate its presence in the relevant presynaptic nerve endings. Also, it was essential to establish that it is actually released when the nerve is stimulated electrically.

One of the main problems confronting all ideas concerning chemical transmission was the instability of the transmitter substance acetylcholine. This had already been synthesized in the mid-nineteenth century. René de M Taveau, previously associated with J. J. Abel, showed that of 20 derivatives of choline, the acetyl ester is the most active in reducing heart rate and blood pressure, an effect opposed by atropine (**Hunt and Taveau, 1906**). It was first isolated from natural sources in 1914 by Arthur
Ewins, a member of the laboratory of Henry Dale as a component present in an extract of ergot. At the time of his appointment to a post at the Wellcome Physiological Laboratories in 1904, Dale remarks that his employer requested that

when I could find an opportunity for it without interfering with plans of my own, it would give him a special satisfaction if I would make an attempt to clear up the problem of ergot, the pharmacy, pharmacology and therapeutics of that drug being then in a state of obvious confusion... I was, frankly, not at all attracted by the prospect of making my first excursion into (pharmacology) on the ergot morass. Dale (1953)

**ERGOT**

Although recognized as the “noxious pustule in the ear of grain” over 2500 years ago, written descriptions of ergot poisoning did not appear until the Middle Ages. Ergot is a fungal parasite, *Claviceps purpurea*, that affects grains, particularly rye. The symptoms of ergot poisoning include hallucinations, burning pains in the limbs followed by gangrene, the tissue becoming dry and being consumed by the “Holy Fire, blackened like charcoal.” Gangrene is the consequence of prolonged vasoconstriction of peripheral vasculature. Further complications included abortion and convulsions. It has been suggested (Camporesi, 1989) that the St Vitus dance, best described as rave parties of the Middle Ages, may have been a manifestation of the seasonal starvation that occurred during the early summer months. This was the time of the “hungry gap” when the grain stocks were awaiting replenishment with the August harvests. The disease was especially tragic as it was most likely to strike the poorest individuals and intensified the worst harvests—rye is extremely cold-tolerant and was, for many Europeans, food of last resort. Relief was obtained at the shrine of St Anthony, where, perhaps fortuitously, sufferers received a diet free of contaminated grain. Today, the most well-known components of ergot must be the hallucinogen, lysergic acid diethylamide (LSD), and ergotamine, which is sometimes used in the relief of migraine (because of its vasoconstrictive properties).

For Henry Dale the extracts of ergot presented a veritable cornucopia of active substances, to which he returned repeatedly over several years. It was an impurity in a sample of ergot sent to him in 1913 for routine quality control (probably due to contamination by *Bacillus acetylcholini*: fresh ergot does not contain acetylcholine) (see Bacq, 1975),
that led him back to the question of transmission at the contacts between nerves and cells. When injected into the vein of an anesthetized cat, the extract caused profound inhibition of the heart beat and being obviously unsuitable for release as a drug, he obtained the whole batch for further investigation in his laboratory. The first thoughts were that the active constituent might be the stable compound muscarine, but on isolation it was found to be the profoundly labile acetylcholine. In a letter to Elliott he writes:

We got that thing out of our silly ergot extract. It is acetylcholine and a most interesting substance. It is much more active than muscarine, though so easily hydrolysed that its action, when it is injected in the blood-stream, is remarkably evanescent, so that it can be given over and over again with exactly similar effects, like adrenaline (the subject of Elliott’s research, see section about the receptive substance). Here is a good candidate for the role of a hormone related to the rest of the autonomic nervous system… Letter, Dale to Elliott, December 11, 1913, Contemporary Medical Archives Centre, Wellcome Institute, GC/42 “T.R. Elliott,” quoted in Bennet (2000).

**Eserine** or physostigmine, an alkaloid isolated from the Calabar bean, had previously shown by Loewi to be an acetylcholinesterase inhibitor.

**John Eccles**, awarded the Nobel Prize together with Andrew Huxley and Alan Hodgkin in 1963 for discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane.

At that time, and even later when it was identified as a chemical component in nonneural tissues, there was never a hint that acetylcholine might have physiological functions. This was not even suggested by the finding as late as 1930 that arterial injections of acetylcholine could induce contractures in denervated muscles. There were still a number of real problems to be overcome. Chief among these was the transient nature of the pulse of neurotransmitter. Also, it was not sufficient to show that acetylcholine applied from a pipette was capable of inducing a response. To prove its role in neurotransmission, it still remained necessary to demonstrate its presence in the relevant presynaptic nerve endings and then to show that it is released upon electrical stimulation.

Feldberg describes his introduction of eserine and the use of a leech-muscle preparation treated with eserine as a specific and sensitive device
for measuring the acetylcholine present in the various effluents (blood, organ perfusate, etc.). This was the key that opened the way to the eventual conversion of that most obdurate, skeptic electrophysiologist John Eccles. Even so, without naming any names, Zenon Bacq (1975) reports that even as late as 1950, certain eminent physiologists were still refusing to incorporate the theory of chemical transmission into their teaching.

**Hormone-inspired robots**

The wisdom brought to light by the proto-endocrinologists, and later expanded by thousands of endocrinologists, has not only led to a thorough understanding of how organisms function and how errors in cellular communication lead to disease, it also provided a rational approach for the development of a rather clever, hormone-inspired, self-reconfigurable robot. CONRO, for configurable-robot, is developed at the ISI institute of the University of Southern California. It is made of separate but connected modules, each operating in its own way, but at the same time having the capacity to cooperate and perform locomotion and self-reconfiguration tasks (see Figure 1-9 for two different configurations). They achieved this by developing an adaptive communication model that mimics the essential properties of hormone-based signaling (a nice example of biomimetics). According to Wei-Min Shen and Peter Will, from a computational point of view, a hormone signal is similar to a content-based message but has the following unique properties: (1) it has no specific destination, (2) it propagates throughout a network, (3) it may have a lifetime, and (4) it may trigger different actions for different receivers (depending both on

![FIGURE 1-9](CONRO, a hormone-inspired self-reconfigurable robot. A modular self-reconfigurable robot has been developed by mimicking the concept of hormone-based signaling. Note the two different configurations: on the left a butterfly-like configuration, allowing rapid movement, on the right a snake-like, allowing passage through very narrow spaces (but at a slow pace).)
specific receptor-coupled effector mechanisms and on the local context, like signals from other sensors and the state of the module). Biologists could not have bettered the computer scientists’ accurate description of hormone action (Shen et al., 2002, 2004).

For a brief but revealing video of how complex schemes of stimulus-response coupling can lead to rather sophisticated behavior of robots: http://www.isi.edu/robots/movies/MankinCONROWeb.mov.

THE RECEPTIVE SUBSTANCE

As the list of mediators grew, hormones and neurotransmitters, the question followed how they act on sensitive tissues. Important progress in this matter came from two lines of research, one from the field of immunology, the other from physiology. Essential is the notion that cause and effect is the consequence of molecular interaction; the action of medications or hormones is a manifestation of their specific “affinity” for particular constituents of cells. In the words of Paul Ehrlich “corpora non agunt nisi fixate,” translated as “a component cannot act if it is not bound.”

When Ehrlich took on medical education, around 1870, Germany was at its zenith of colonial expansion, and confronted with nasty diseases in far-flung outposts. Moreover, industrial preparation of textile dyes had just become a booming business. Companies like Hoechst, AGFA, and BASF took their origin in dye production, in particular those derived from aniline, a product obtained from distillation of coal tar (creosol). Aniline and methylaniline (toluidine) had become fashionable compounds when, in 1856, William Henry Perkin (an 18-year student of August Hofmann at the Royal College of Chemistry) had produced a purple colorant known under the name of aniline purple. Microscopists too became interested in these dyes as a way to distinguish between cells or cellular organelles. Among these was Ehrlich, a medical student, who wrote his doctorate dissertation on the subject of “Contributions to the Theory and Practice of Histological Staining” (Figure 1-10). One of the most outstanding results of his doctorate investigations was the discovery of the mast cell, rich in heparin-containing granules that bind toluidine blue. Thanks to the application of textile dyes, a subdivision of granulocytes was discovered, those that strongly stained with eosin (eosinophils), those that stained with hematoxylin (basophils), and those that hardly stained at all (neutrophils). At a later stage, Ehrlich also developed an improved staining for the freshly discovered tubercle bacillus (Mycobacterium tuberculosis, Robert Koch in 1882) using aniline, fuchsine, and gentian violet (Sakula, 1882).

The word goes that the cell specificity of staining had instigated Ehrlich’s idea that textile dyes could possibly be used in chemotherapy. Here we employ the term in its original broad sense: the use of synthetic
chemicals working together with the body’s own defenses to combat infection or new growth (neoplasia). Thus he showed that trypan red has some effect on *Trypanosoma equinum* (but not on the human pathogens *brucei* and *cruzi*). Methylene blue was shown to block gametocyte development of *Plasmodium falciparum* and was introduced as an antimalaria agent (*Adjalley et al.*, 2011). It eventually eclipsed because soldiers fighting in the Pacific battleground of World War II strongly disliked its prominent adverse effects: it turned their eye balls blue and their urine green. Two very successful medicaments are a direct, although being delayed, outgrowth of Ehrlich’s postulation, namely prontosil (antibacterial agent obtained from the red dye sulfonamidochrysoidine) and suramin (an antityrpanosomiasis compound derived from the trypan dyes) (*Wainwright*, 2010). A last example, unrelated to Paul Ehrlich, Paris green, an arsenite-containing dye, had been successfully employed to control the Colorado potato beetle back in 1867.

Perhaps most noticeable is Ehrlich’s contribution, together with Sahachiro Hata, to the discovery of a first treatment against syphilis. In a long and highly systematic search for compounds that cured trypanosome infection (sleeping sickness) in mice, they found an effective organic arsenic compound named arsphenamine (number 606 in a range of over 915 compounds scrutinized). It was Hata’s contribution to show that it was much more effective against *Treponema pallidum*. Within 1 year of discovery, it was commercialized by Hoechst under the name of Salvarsan. Indeed, an “arsenic salvation” it must
have been for all those suffering from syphilis. The compound rapidly made its fame (or ill fame, depending on how much one suffered from its adverse effects) until superseded by penicillin in the 1940s.

In his Nobel Lecture (1908) Ehrlich joins rank with Thomas Huxley when he proclaims:

…that for a further penetration into the important, all-governing problem of cell life even the most highly refined optical aids will be of no use to us. Now, at this moment, the time has come to penetrate into the most subtle chemism of cell life and to break down the concept of the cell as a unit into that of a great number of individual specific partial functions. But since what happens in the cell is chiefly of a chemical nature and since the configuration of chemical structures lies beyond the limits of the eye’s perception we shall have to find other methods of investigation for this. This approach is not only of great importance for a real understanding of the life processes, but also the basis for a truly rational use of medicinal substances.

PRONTOSIL

Sulfonamidochrysoidine, a red dye, was first synthesized by Bayer chemists Josef Klarer and Fritz Mietzsch as part of a research program designed to find dyes that might act as antibacterial drugs in the body. The molecule was tested and in the late autumn of 1932 was found effective against some important bacterial infections in mice by Gerhard Domagk. It was marketed as prontosil and is now considered being the first effective antibiotic (with more or less acceptable adverse effects). In late 1935, the group of Ernest Fourneau, at the Institut Pasteur in Paris, showed that prontosil becomes active only after metabolic processing in which sulfanilamide is produced. This specifically blocks folate production in bacteria, hence preventing their DNA synthesis (acting as bacteriostatic agent). Sulfanilamide served as a lead for the production of new sulfonamide antibotics of which sulfamethoxazole is still in use (component of co-trimoxazole).

DYES AND NAMING MICROBES

To illustrate once again how much dyes influenced the scientific enterprise at the end of the nineteenth century, T. pallidum was thus named because of its pallid appearance. The bacterium proved to be difficult to stain with textile dyes. Current diagnosis protocols include dark-field microscopy, immunostaining (Treponema-specific antibodies), polymerase chain reaction, and, sometimes, a rabbit infectivity test.
However, it was during his studies on therapeutic sera (starting around 1890), in particular dealing with a treatment for diphtheria, that Paul Ehrlich elaborated the idea of a “receptive side chain.” He reasoned that the sera (which he obtained through collaboration with Emil von Behring) must contain substances that first bind with and subsequently inactivate bacterial toxins. These substances have entered the serum, after an animal or human is exposed to the pathogenic agent. To explain how they came about, Ehrlich proposed that certain membrane proteins, which he named “side chains” of a particular cell have an “atom group” that, by mere coincidence, possesses specific combining properties for a particular toxin. Upon binding, both the side chain and the toxin lose their activity. In order to make up for the loss of activity, the cell starts to produce large amounts of the side chain. Many of the excess side chains can now break off and are released into the blood stream, thus acting as soluble antitoxins; hence the protective action of serum (the soluble component of blood after clotting). He assumed that the antipodes, toxin and receptive side chain, engage in a chemical bond, albeit reversible, through the principle of “lock and key” (a comparison made by Emil Fischer). We must credit Ehrlich not only for his systematic approach to screening of medicaments but also for his excellent sense of imagination. Although his theory does not rhyme with our current understanding of immunity, receptor targets for toxins and neutralizing immunoglobulins (antibodies) are quite distinct entities, Ehrlich’s theory of receptive side chains provided a strong impulse for the development of vaccination and passive immunization protocols. Furthermore, it opened the way to search for toxin receptors, the target by which they interact with the infected organism and cause disease.

Quite surprisingly, Ehrlich initially refused to apply the receptive side chain theory to his earlier studies with dyes. He argued that the easy
reversibility of staining (removed by alcohol, acetone, or water) made it unlikely that the dyes bound “receptive side chains” (Croonian lecture, 1900). He gradually changed his mind while reading about Langley’s “receptive substance,” which bound even smaller molecules like nicotine. Around 1907, Ehrlich introduced the words “chemoreceptor” and “poison receptor” or just “receptors” (Nobel Lecture, 1908). The passage below, from his Nobel Lecture, witnesses how much Ehrlich’s vocabulary resembled that of the Langley (or how two disciplines, immunology and physiology had converged to point to a unifying concept):

when we see how some bacterial poisons produce disturbance only after weeks of incubation and then damage the heart or the kidney or nerves, when we see how animals suffering from tetanus present contractions and spasms for months, we are forced to the direct conclusion that all these phenomena can only be caused by the adhesion of toxic substance to quite definite cell complexes. I therefore assumed that the tetanus toxin for instance, must unite with certain chemical groupings in the protoplasm of cells, particularly the motor ganglion cells, and that this chemical union represents the prerequisite and cause of the disease. I have therefore simply called such groupings “poison receptors” or just “receptors.”

Ehrlich shared the Nobel Prize in Physiology or Medicine in 1908 with Ilya Mechnikov “in recognition of their work on immunity.”

Gerhard Domagk received the Nobel Prize in Physiology or Medicine in 1939 “for the discovery of the antibacterial effects of prontosil.”

Emil von Behring received the Nobel Prize in Physiology or Medicine in 1901 “for his work on serum therapy, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths.”

John Newport Langley, who remained throughout his whole career at the University of Cambridge, took over where Claude Bernard, professor in medicine at “College de France” in Paris, had left the stage, namely, to sort out where exactly did curare and nicotine (or atropine and pilocarpine) act in the stimulus-response coupling between nerves and striated (or smooth) muscle (Figure 1-11)? Bernard had made the crucial observation that when frog muscles are paralyzed by curare, they fail to respond to electric stimulation of the motor nerve but they still contract when the current is applied directly to muscle. In order words, the contractile machinery of actin and myosin is not affected by curare (Maehle et al., 2002; Bennet, 2000).
He approached the question in different experimental settings over a period of 30 years, with an interlude of 20 years during which he investigated the secretion of pepsinogen and pepsin. Concerning stimulus-response coupling, following an assignment by his supervisor Professor Michael Forster, Langley commenced his career with the elucidation of the effects of the drug jaborandi (pilocarpine) in frog hearts and submaxillary glands from cats and dogs. This short prelude already made him conclude that pharmacological agents form compounds (chemical groupings in the words of Ehrlich) with a substance in cells. In his article in 1878 he comments (Langley, 1878):

we may, I think, without much rashness, assume that there is a substance or substances in the nerve endings or gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption then the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors.

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**FIGURE 1-11**  Langley’s mission: Where does tubocurarine affect the neuromuscular transmission? (a) Acetylcholine and noradrenaline as transmitters in the peripheral nervous system. CNS, central nervous system; PVGC, paravertebral ganglion chain; Ach, acetylcholine; nic, nicotinic; mus, muscarinic; NA, noradrenaline. (b) Microelectrode recording at the end plate (1) and away from the end plate (2) in the muscle fiber. Tubocurarine reduces the end plate potential so that no action potential is generated (muscle paralyzed). (c) Langley had to sort out where exactly tubocurarine acted. We now know that tubocurarine blocks the binding of acetylcholine to its receptor (the nicotinic acetylcholine receptor located in the postsynaptic membrane (sarcolemma)). Image of muscle fibers from Dr T Caceci (Virginia–Maryland College of Veterinary Medicine).
ORIGIN OF THE TERM (ORTHO) SYMPATHETIC AND PARASYMPATHETIC

It was Jacob Benignus Winslow (1669–1760), Danish-born Parisian anatomist, who in 1716 renamed the “intercostal nerve” (now called para-vertebral ganglion chain) to “great sympathetic nerve.” In fact, because of its numerous communications with most of the major nerves of the whole body, this structure was considered by Winslow as being “sympathetic” with other neural structures. Subsequently, Langley introduced the term “parasympathetic innervations” in order to distinguish the cranial nerves (among which the vagus nerve) from the sympathetic ones. Sympathy, both neural and humoral, is an old notion, it accounts for the cooperation or coordination of organs.

THE SOMATIC AND AUTONOMIC NERVOUS SYSTEM

For a concise overview of the somatic and autonomic nervous system, its chemical mediators and the numerous medicaments that affect their function, we suggest Chapters 9–11 of “Pharmacology” (Rang et al., 2007).

CURARE

Curare is mixture of naturally occurring alkaloids found in various South American plants and used as arrow poisons. The most important component is tubocurarine, the structure of which was elucidated in 1935 (purified from Strychnos toxifera). Tubocurarine qualifies as nondepolarizing blocking agent: it acts as a competitive antagonist of the nicotinic Ach receptor in the postsynaptic membrane of the end plate.
Roughly 20 years later, Langley returns to the subject of nervous transmission this time in the context of the efferent somatic system (motor neurons) and with the results of Elliott firmly in the back of his mind. Thomas R. Elliott, a student from Langley, had shown that adrenaline (or suprarenal extracts) led to contraction of smooth muscle (intestinal tract) in a similar fashion as did stimulation of the sympathetic nerves. The action persists even when the sympathetic neurons were degraded and he concluded that “adrenaline might then be the chemical stimulant liberated on each occasion when the (nerve) impulse arrives at the periphery” (Elliott, 1905). In short, Elliott proposed that adrenaline, which qualified as a hormone, could also act as a neurotransmitter. We now know that this is noradrenaline (see Figure 1-11(a)).

Langley applied similar experiments to anesthetized fowl and measured contraction of the gastrocnemius muscle following injection of nicotine into the bloodstream. In order to remove nerve innervations, the same experiments were repeated with animals from which the sciatic and crural nerves were cut weeks in advance (leading to full degeneration). Upon injection of nicotine, the animals stretch their legs (and open their eyes) and this is undone by the subsequent injection of curare (Figure 1-12). However, direct electric stimulation of the same muscle still leads to contraction. “It follows then that curare acts upon the muscle substance and not upon the axon-endings. Since, in the normal state, both nicotine and curare abolish the effect of nerve stimulation, but do not prevent contraction from being obtained by direct stimulation of the muscle or by further adequate injection of nicotine, it may be inferred that neither the poisons nor the nervous impulse act directly on the contractile substance of the muscle but on some accessory substance. Since this accessory substance is the recipient of stimuli which it transfers to the contractile material, we may speak of it as the receptive substance of the muscle” (Bennet, 2000; Langley, 1905).
Apparently, Langley disliked speculating and developing hypothesis and he has left no graphical representations of his (private) mental pictures. Had he be forced to draw his receptive substance in relation to the contractile apparatus, in the way Ehrlich has done for the relationship between toxin and the side chain (Figure 1-10), we would have learned that the proposed mechanism was far from correct. Langley only gives a hint about a possible mode of action in 1908 by stating:

My theory of the action is in general in lines of Ehrlich’s theory of immunity. I take it that the contractile molecule has a number of “receptive” or “side-chain” radicles and that nicotine, by combining with one of these, causes contraction and, by combining with another, causes twitching… Bennet (2000)

The term “receptor” does not yet appear in the index of the 1955 edition of The Pharmacological Basis of Therapeutics, the standard American textbook of pharmacology, but the following sentence was there: “Years ago, Langley named the differentiating substance the ‘receptive substance’; this term is still widely employed, but it must be realized that the ‘receptor’ may not be a morphologically demonstrable structure” (Goodman and Gilman, 1955; Nobel speech AG Gilman, 1994). Perhaps, that is why Langley was often nominated but never rewarded a Nobel Prize for his discoveries and so we pay our tribute to his pioneering work with a fragment from his obituary:

Each gain he made was a step placed securely and finally and few indeed of them, as the road has become form firmly and widely trodden by others following, have been found wrongly placed. All his chief words keep, and must always keep their place in the significant history of Animal Physiology. The bare titles of his papers and books deployed there along the years give the plainest testimony to me to his unvarying, unhalting service to science. No single year in all that series, extending will nigh for half a century, from youth to age, appears without its contribution of effective work. Fletcher (1926)

More importantly of course is that Langley’s concept of the receptive substance was taken up by others, notably Dale and Loewi with respect to chemical synaptic transmission. By 1970, the nicotinic acetylcholine receptor was purified from the electric organ of Torpedo (Miledi et al., 1971; Changeux et al., 1970). In that year Katz and Miledi, using micropipettes, demonstrated that addition of acetylcholine was linked to changes in membrane conductance in single synapses (Katz and Miledi, 1970). It took longer for the adrenergic receptor, as there are no tissues that express it so abundantly as the nicotinic acetylcholine receptor in Torpedo, but by 1984 it was shown that reconstitution of the pure receptors with G-proteins and adenylyl cyclase was sufficient to create a neurotransmitter responsive system (Cerione et al., 1984) and by 1986 the first mammalian beta-adrenergic receptor was cloned (Dixon et al., 1986).
PROTO-MESSENGERS AND -RECEPTORS

It is obvious that the very first stimulus to a presumed primordial organism must have been from the immediate environment, signals that hinted to the presence or absence of conditions, which allowed or prevented replication. Bacteria have developed sophisticated chemotactic responses to nutrients, connecting membrane receptors with flagellar motors (Bren and Eisenbach, 2000). Molecules that we now regard as messengers (extra- or intracellular) of our bodies are of great antiquity on the biological timescale. It is interesting to consider which came first: the messengers or the receptors that they control? The general view is that the messengers, and their pathways for biosynthesis, preceded the development of concomitant receptors as we know them in mammals. An explanation for this order of appearance may be explained by an as yet hypothesis that receptors occurred as a consequence of convergence of fragments of genes involved in metabolic pathways. For instance, it has been shown that the human folate receptor-α (FOLR1), a membrane transport protein, carries sequence homology with several enzymes involved in folate synthesis in Saccharomyces cerevisiae (among others: polyglutamate synthase, folic acid synthesis protein FOL-1, dihydrofolate reductase, GTP cyclohydrolase 1, and folylpolyglutamate synthase) (Ramamoorthy and Shanker Verma, 2008). There is a sense of logic in that, like receptors, metabolic enzymes too will have to recognize the metabolites and one may assume that the number of binding pockets (suitable biochemical space) that nicely fits folate (or an intermediate product) is limited. These newly formed binding sites then connect with signaling or transporter activities and new functions appear that are entirely unrelated to the metabolic pathways (although they may affect them in one way or another).

**Folate**, from Latin folium, is present in the leaves of fresh vegetables. Folate (folic acid or water-soluble vitamin B9) is an essential component for nucleotide synthesis (AMP, GMP, TMP) and amino acid metabolism (methionine, histidine, serine). Humans have lost the capacity to synthesize folate and rely on external sources. Deficiency during embryonic development is associated with neural tube defects (spina bifida and anencephaly) (OMIM 601634).

Substances recognized as having the actions of hormones in animals first made their appearance at early stages of evolution. For instance, chemical structures closely related to thyroid hormones have been
discovered in algae, in sponges, and in many invertebrates. Steroids such as estradiol are present in microorganisms and also in ferns and conifers. Catecholamines (dopamine and (nor)adrenaline) have been found in protozoa (Janakidevi et al., 1966a,b) and ephedrine, resembling both adrenaline and amphetamine, can be isolated from the stems and leaves of the Chinese herb ma huang (Ephedra sinica). What it does to the herb is not described but in humans it causes vasoconstriction (peripheral effect, stimulating the action of adrenaline) and because it readily crosses the blood–brain barrier, it qualifies as an illegal psychostimulant substance (acting as an amphetamine, liberating dopamine and noradrenaline in certain neural synapses). There are claims, based on immunological detection, for the presence of peptides related to insulin and the endorphins in protozoa and fungi (LeRoith et al., 1980) although no signal function has been discerned.

The α- and α-type mating factors of yeast (produced by α- and α-cells, respectively), which certainly act as messengers, are very similar in structure to gonadotropin-releasing hormone that controls the release of gonadotropins from the anterior pituitary in mammals (LeRoith et al., 1983; Hunt and Dayhoff, 1979). Factors resembling the mammalian atrial natriuretic peptide are present in the cytosol of the single cell eukaryote Paramecium multimicronucleatum (Vesely and Giordano, 1992) and in the leaves of many species of plants where they act as regulators of solvent and solute flow and of the rate of transpiration (Vesely et al., 1993). ACTH and β-endorphin are present in protozoa. These organisms also contain high MW precursors of these peptides reminiscent of the vertebrate proopiomelanocortin (LeRoith et al., 1981, 1982).

Receptor-like proteins in nonanimal cells have been much harder to identify. A recently described example is a protein expressed in the plant Arabidopsis that shares extensive sequence homology with the ionotropic glutamate receptor of mammalian brains (Lam et al., 1998). A corollary arising from this is the possibility that the potent neurotoxins thought to be generated in defense against herbivores may have their origin as specific agonists and were only later selected and adapted in some species as poisons. GABA_B-like receptors and Frizzled-like receptors (for the messenger Wnt), which were thought to be exclusive for the animal kingdom, have been detected in slime molds (Dictyostelium discoideum). The corresponding messengers remain elusive on this occasion (Prabhu and Eichinger, 2006). Molecules having a close relationship to the receptors for epidermal growth factor (EGF) and insulin apparently evolved in sponges prior to the Cambrian Explosion (more than 600 million years ago) and it has been proposed that they may have contributed to the rapid development of the complex multicellular phyla (Skorokhod et al., 1999).
Although invertebrates express some members of the nuclear receptor family (such as the receptor for thyroid hormone and vitamin D), nuclear receptors for adrenal and sex steroid hormones (cortisol, aldosterone, testosterone, estradiol, progesterone, etc.) are absent (Escriva et al., 2003). The ancestral steroid hormone receptor probably made its first appearance in a cephalochordate such as amphioxus. Receptors for estradiol, progesterone, and cortisol have been cloned from lamprey (fish-like, considered an ancient lineage of vertebrates). From hereon in evolution the steroid hormones would have allowed for a ligand-based mechanism for the regulation of gene transcription and this could have promoted the complex processes of differentiation and development found in the higher vertebrates (Baker, 2003). Thus, the hox genes that are critical for development and differentiation, including the brain of amphioxus (Manzanares et al., 2003) are regulated by estrogens and progestins.

An important consequence of the separate arrival of messenger and receptors in the course of evolution is that the responses to a given messenger (be it a hormone or other) can vary widely across different species and even within species. Two examples: numerous actions of prolactin have been identified (Nicoll, 1982). It is the regulator of mammary growth and differentiation and of milk protein synthesis in mammals. In birds, it acts as a stimulus to crop milk production and in some species as a controlling factor for fat deposition and as a determinant of migratory behavior (Meier et al., 1965; Meier, 1977). It is a regulator of water balance in urodeles (newts and salamanders) and of salt adaptation and melanogenesis in fish (Nicoll, 1982). Serotonin (5-hydroxytryptamine), a neurotransmitter that controls mood in humans is reported to stimulate spawning in mollusks, probably as a consequence of its conversion to melatonin (naturally, one wonders whether it affects their mood as well). These examples highlight an important takeaway message, signaling processes have not been designed to serve certain functions, acting in defined
stimulus-response circuits; they arose by chance, provided new opportunities (new ecological niches, increased survival, or others) and therefore stood the test of time. Pathways may thus have appeared independently in different species, they may have been transmitted between species through gene transfer mechanisms or they were inherited from ancestors. Certain receptors or signaling components may have disappeared in one species but remained opportune in others. An evolutionary reconstruction of signaling pathways may reveal how they came about, and disappeared, but this lies beyond the scope of this book (see, for instance, Anantharaman et al. (2007) and Schaap (2011)).

**GROWTH FACTORS: SETTING THE FRAMEWORK**

The trails that led to the discovery of the growth factors (and related messengers) are very different from those that revealed the first hormones and neurotransmitters. For a start, people knew, more or less, what to look for and where to go looking, and in general, the tale is somewhat less romantic and less fraught with angst and vehement disagreements. However, what began with a simple search for factors that would sustain living cells in laboratory conditions has expanded into a plethora of subject areas, which are subjects in their own right. These include inflammation, wound healing, immune surveillance, development, and carcinogenesis. To confront this bewildering prospect, we first set out some details of how it evolved initially so that the reader is aware of how the major questions developed and have been confronted. A difficulty arises from the convergence of different disciplines, each bringing with it the baggage of its favored nomenclature. We return to this matter at the end of this chapter.

**Viruses and tumors**

The first report of a tumor linked to a virus appeared in 1908, when Ellermann and Bang obtained a filterable agent from a chicken leukemia and were able to make six passages of it, from fowl to fowl, producing the same disease each time (Ellermann and Bang, 1908). Their report was generally disregarded. Leukemia was not considered to be a tumor, though as should have been evident from the work of Aldred Warthin (Figure 1-13), first reporting in 1904 (Warthin, 1904, 1907), a link between leukemias and tumors, and hence cell growth and proliferation, had already been established:

“These conditions are comparable to malignant tumors. The formation of metastases, the infiltrative and destructive growth, the failure of innoculations and transplantations etc., all favor the view that they are neoplasms, and present the same problems as do the malignant tumors.”
More than 20 years were to pass before the leukemias were eventually recognized as being tumors or neoplastic diseases.

Francis Peyton Rous (1910) described a chicken tumor, identified as a sarcoma, that could be propagated by transplanting its cells, these then multiplying in their new hosts giving rise to tumors of the same sort. The cells yielded a virus, now known as Rous sarcoma virus, from which, in 1980, the first protein tyrosine kinase, v-Src, was isolated (Erikson et al., 1980; Hunter and Sefton, 1980). Writing at the end of his career in 1967, Rous gives an apt description of how things were done:

Those were primitive times in the raising of chickens. They were sold in a New York market not far from the institute, and many individual breeders brought their stock there. Every week F. S. Jones, a gifted veterinarian attached to my laboratory, went to it, not only to buy living chickens with lumps which might be tumors, but any that seemed sickly and had a pale comb, as perhaps having leukemia. Thus within less than four years we got more than 60 spontaneous tumors of various sorts…

FIGURE 1-13  Stained blood smear from a leukemic fowl. “In December 1905, there came into my hands a Buff Cochin Bantam hen showing signs of illness in the way of indisposition to move about and a general weakness of a progressive character. No symptoms of ordinary fowl diseases were present… Examination of blood smears showed, however, a great increase of white cells of the large lymphocyte type… A diagnosis of leukemia was therefore made… A great variety of staining methods were used, including the most recent methods for the staining of spirochetes and protozoan parasites… No evidence of the existence of any infective agent could be obtained” (Warthin, 1907). (Note that avian red blood cells (yellow stain) are nucleated.)
Francis Peyton Rous (1879–1970) shared the 1966 Nobel Prize for Physiology or Medicine for his discovery of tumor-inducing viruses.

Sarcoma is a form of cancer that arises in the supportive tissues such as bone, cartilage, fat, or muscle. A carcinoma is defined as a malignant new growth that arises from epithelium, found in skin or, more commonly, the lining of body organs, for example: breast, prostate, lung, stomach, or bowel. Carcinomas tend to infiltrate into adjacent tissue and spread (metastasize) to distant organs, for example: to bone, liver, lung, or the brain.

The discovery of nerve growth factor…and EGF

Among the fractions that I assayed in vitro the following day, there was one containing snake venom. Having not been told which of the fractions had been specially treated, I was completely stunned by the stupendous halo radiating from the ganglia. I called Stan in without telling him what I had seen. He looked through the microscope’s eyepieces, lifted his head, cleaned his glasses which had fogged up, and looked again. “Rita,” he murmured, “I’m afraid we’ve just used up all the good luck we’re entitled to. From now on, we can only count on ourselves…” Events were to prove him wrong.

v indicates a viral origin, commonly a transforming mutant. c indicates the cellular wild type. Src, sarcoma.

Nerve growth factor (NGF) may perhaps be regarded as the first identified growth factor, but there were many early clues hinting at their existence. The embryologist Hans Spemann (Nobel Prize, 1935) had described the eponymous “organizer” that directs the creation of the dorsal axis (development of neural tube, notochord, vertebral discs) in the gastrula stage in the development of the amphibian embryo (see Chapter 17). This work had famously indicated that soluble factors made by the embryonic cells must be instrumental in regulating cell proliferation and differentiation (Hamburger, 1969).
Rita Levi-Montalcini’s affair with growth factors had its origin at that time. Dismissed from her university post by the Nazi racial edicts, she determined to continue on alone. She had been alerted to the work of Spemann by an article written by one of his protégés, Viktor Hamburger. He described the concept of the inductive reaction of certain tissues on others during early development. In particular, he cited the effect of ablating the embryo limb buds of chicks upon the reduction in the volume of the motor column and the spinal ganglia responsible for the innervation of the limbs. The idea was that the failure of the cells to differentiate and to develop is due to the absence of an inductive factor normally released by the innervated tissues. She aimed to understand how the excision of noninnervated tissue could affect differentiation and subsequent development.

Stanley Cohen and Rita Levi-Montalcini were awarded the Nobel Prize in 1986 “for their discoveries of growth factors.”

Confined to working in her bedroom she made use of just the most basic materials and instruments needed for histological investigation. In her examination of this problem, Levi-Montalcini found that nerve cell differentiation proceeds quite normally in the embryos with excised limbs, but that a degenerative process (what we would now call apoptosis) commences as soon as the cells emerge from the cord and ganglia appear at the stump of the amputated limb (Hamburger and Levi-Montalcini, 1949; Levi-Montalcini, 1950). It appeared to her that the failure to develop was best explained by the absence of a trophic factor.

In 1946, she sailed for the USA in the company of Renato Dulbecco (see below), a friend from her student days. She was destined for St Louis, he for Bloomington. Intending to pay a brief visit of a few weeks to the laboratory of Viktor Hamburger, she remained there for 30 years. The work that led eventually to the discovery of NGF had its origins in the observation by a late student of Hamburger’s, Elmer Bueker. He had reported that fragments of an actively growing mouse tumor, grafted on to chick embryos, caused a great ramification of nerve fibers into the mass of tumor cells (Bueker, 1948). Seeking advice and encouragement, he suggested that the tumor generated conditions favorable for the differentiation of the...
nerve cells, which was reflected in the increased volume of the ganglia. Repeating the experiment, Levi-Montalcini describes the extraordinary spectacle of seeing bundles of nerve fibers passing between the tumor cells like rivulets of water flowing steadily over a bed of stones. In no case did they make any connection with the cells, as is the rule when fibers innervate normal embryonic or adult tissue. Later she describes how the sympathetic fibers invaded the embryonal viscera, even entering into lumen of the venous, but not the arterial blood vessels so that the smaller veins were quite obstructed.

The penetration of the nerve fibers into the veins, furthermore, suggested to me that this still unknown humoral substance might be exerting a neurotropic effect, or what is known as a chemotactic directing force, one that causes nerve fibers to grow in a particular direction... Among these, I guessed, was undoubtedly also the humoral growth factor that the cells produced. This hypothesis would explain this most atypical finding of sympathetic fibers gaining access inside the veins... Now, with the hindsight of the nearly forty years gone by since those moments of keenest excitement – it appears that the new field of research that was opening up before my eyes was, in reality, much vaster than I could possibly have imagined.

It was clearly necessary to develop an *in vitro* assay. These were early days in the field of cell culture and it took the best part of 6 months to develop a practical method that could be used as the basis for measuring the biological activity of fractions in protein purification. Only then was the point reached when a biochemical approach could usefully be applied in the pursuit of NGF, the name by which the factor became known shortly after.

“Rita,” Stan said one day, “you and I are good, but together, we are wonderful.”

After a year’s intense work, they had narrowed down the factor as a nucleoprotein, though Stanley Cohen suspected that the nucleic acid component was likely to be a contaminant. On the advice of Arthur Kornberg, he applied an extract of snake venom as a source of nuclease activity with the aim of removing nucleic acids, present as an impurity in their material. To their great surprise, this yielded a preparation that enhanced neuronal growth still further. It emerged that the snake venom alone was active (*Cohen and Levi-Montalcini, 1958*). Making the connection between venom and saliva, they tested mouse salivary glands and found that this too is an excellent source of NGF activity. (If they had been able to purchase purified nuclease enzyme, the course of the discovery must surely have been prolonged.)

Later, Cohen discovered a new phenomenon that “was destined to become a magic wand that opened a whole new horizon to biological studies.” A contaminating factor was present that caused precocious
growth in epidermis (Figure 1-14). It was also found that the factor has a powerful proliferative effect on connective tissues and it became clear that there is a link between the mechanisms that control normal proliferation and neoplastic growth.

Since, under culture conditions, the stimulus to proliferate could not involve systemic or hormonal influences, Cohen called the new protein EGF (Cohen, 1964, 1986). Later, it was shown that mouse EGF enhances DNA synthesis in cultured human fibroblasts. It was also found that EGF is similar to urogastrone, a peptide that had been isolated from human urine and recognized by pharmacologists because of its ability to inhibit gastric acid secretion (Gregory, 1975). Out of 53 residues in the amino acid sequence, 37 share a common location and the 2 polypeptides have similar effects on both gastric acid secretion and the growth of epidermal cells.

All this now paved the way for a more molecular approach using isolated cells. It was found that rat kidney cells, transformed with the Kirsten sarcoma virus fail to bind EGF. This downregulation, which is due to internalization of the receptor, is caused by the elevated expression and release of EGF by the cells themselves (an autocrine mechanism of feedback regulation). The possibility that internalization might be a necessary step initially found many adherents, but it became apparent that the transduction mechanism emanates from events at the plasma membrane. In particular, ligand binding directly induces phosphorylation (on tyrosine residues) of a membrane protein, later shown to be the EGF receptor (EGF-R) itself (Ushiro and Cohen, 1980). This was an important breakthrough because tyrosine kinase activity had already been associated with virus-induced sarcomas (the v-src gene product). Thus, a firm link was established between neoplasia and the physiological regulation of cellular growth.
Further evidence for the role of growth factors in tumor generation came with the revelation that the avian erythroblastosis virus oncogene, v-erb-B, codes a product having similarities with the EGF receptor. Indeed, it became apparent that the transformation derives from inappropriate acquisition from the (cellular) c-erb-B gene, of a truncated receptor, lacking the binding site for EGF and which is constitutively activated (Downward et al., 1984).

The development of this field has generated much excitement but also frustration. It has been exciting, because it has yielded a good understanding of cell transformation and growth factors, and frustrating because it has become clear that cancer cells do not readily lend themselves as specific targets for drugs. The main impetus behind these studies was that nonmammalian genes might be the cause of disease. The hope was to discover targets that might be exploited to kill tumor cells selectively, for example, the products of the viral genes. We now realize that these are initially hijacked from the mammalian genome itself, then inaccurately transcribed by sloppy DNA or RNA polymerases in the virus, which then offers them back to the host upon infection. Apart from this, the large proportion of human tumors is of nonviral origin, arising as a consequence of tumor-promoting substances, radiation, etc.

**Platelet-derived growth factor**

In 1912, Alexis Carrel (Ross and Vogel, 1978) reported a number of experiments having the purpose:

> to determine the conditions under which the active life of a tissue outside that of the organism could be prolonged indefinitely

When he tried to maintain tissues for a few days in a simple buffered salts solution, the cells lost their growth capacity and then their viability. It was supposed that the senility and death of the cultures were due to the accumulation of catabolic substances and exhaustion of essential nutrients. Continuous and more rapid growth was achieved by supplementing the solution with diluted plasma, and then, from time to time, submerging the tissue in serum for a few hours. Interestingly, the notion that the cells might require specific factors present in the serum never appears to have crossed Carrel’s mind. In conclusion, he wrote that

> fragments of connective tissue have been kept in vitro in a condition of active life for more than two months. As a few cultures are now eighty-five days old and are growing very actively, it is probable that, if no accident occurs, the life of these cultures will continue for a long time (Figure 1-15). Hamilton (1986)
Alexis Carrel, the first American to be awarded the Nobel Prize 1912 “in recognition of his work on vascular suture and the transplantation of blood vessels and organs.” He tarnished his reputation by his association with the eugenics movement, calling for the establishment of institutions equipped with “appropriate gases” in order to eliminate the insane. He gave enthusiastic support to the Vichy government during World War II and after the liberation of France he was charged for collaboration with the Nazi occupiers. He died before his case came to trial. Even his claim to have kept heart cells in culture may have been unwittingly fraudulent. Assistants may have introduced fresh cells from time to time in order to achieve the expected result…and to keep Alexis happy: see p. 21 of Hamilton (1986).

About 40 years later, Temin and Dulbecco, working independently, set out to define the precise requirements for cell culture with respect to amino acids, vitamins, salts (together, Carrel’s “nutrients”), and importantly, growth factors. Their ambition to culture cells arose from their interest in the role of viruses in cell transformation and tumor formation. Thus an important landmark was the finding that the requirement for serum is drastically reduced in cells infected with tumor viruses. They proposed that transformation might occur as a result of the enhanced capacity of tumor cells to respond to the proliferation signals present in the serum.
An important turning point was the discovery that serum (the soluble component of clotted blood) supports growth and proliferation. Plasma (the soluble component of blood after sedimentation of cells) merely allows survival and over a period of about 2 days, cells become quiescent (arrested at the G0 stage of the cell cycle). Serum contains the products of activated platelets suggesting that they might have a necessary role in the provision of growth factors. In 1974, Russell Ross showed that factors extracted from platelets can induce quiescent smooth muscle cells to synthesize DNA (Ross et al., 1974), and in the same year, Kohler and Lipton obtained a similar result with mouse 3T3 fibroblasts. A purified factor derived from blood platelets, hence named platelet-derived growth factor (PDGF), could propel the quiescent cells into the cell cycle S-phase. With the purification of PDGF (Antoniades et al., 1979) the question of how it acts to could be faced. PDGF exists as a disulfide linked dimer, so that binding automatically causes the cross-linking of two receptors. This constitutes the signal for activation (Cooper et al., 1982). Subsequently, it was found that the oncogene of the simian sarcoma virus (v-sis) is homologous to the gene coding for PDGF (Doolittle et al., 1983; Waterfield et al., 1983; Robbins et al., 1983). Here was another clear link between a growth factor and a tumor virus. This time, however, the signal to uncontrolled cell proliferation is due to excessive production of growth factor, rather than expression of a constitutively activated receptor. Furthermore, it was shown that v-sis causes cell transformation in primates (Theilen et al., 1971).

The mechanisms by which an oncogene might cause a tumor were becoming clearer. Here is another example of a mammalian gene, surreptitiously borrowed by a virus, then mutated or mutilated. On return to the host by infection, it causes cell transformation and tumor formation.

**Transforming growth factors TGFα and TGFβ**

The transforming growth factors (TGF) were originally isolated from the conditioned medium of a virally infected mammalian fibroblast cell line, 3T3. These are proteins that can bring about transformation of phenotype (Figure 1-16). The discovery of the TGF followed some years after the first reports and descriptions of EGF and it derives particularly from the undertakings in the laboratory of George Todaro (De Larco and Todaro, 1978).
CELL TRANSFORMATION

Fully transformed cells possess one or more of the following characteristics: they may not require exogenous growth factors, they grow in athymic (nude) mice, they are insensitive to contact-inhibition, they can evade programmed cell death (apoptosis), they have limitless replicative potential, they can induce angiogenesis, they no longer require attachment to a substrate (they grow in soft agar), and they may disseminate in different tissues (metastasis).

**FIGURE 1-16** Cell transformation through sarcoma virus-derived growth factors. Fibroblasts cells were brought into culture with (b and d) or without (a and c) medium obtained from sarcoma virus-infected cells (virus-conditioned medium). Note that with conditioned medium (b) cells are no longer contact inhibited and grow in multiple layers and (d) they form colonies in soft agar.
TGFα, although quite distinct from EGF with respect to amino acid sequence, binds to the EGF receptor and signal cells in a similar fashion (see Chapter 10, “Regulation of Cell Proliferation by Receptor Tyrosine Protein Kinases”). A related factor isolated from these tumor cells, TGFβ, which does not compete with the binding of TGFα or EGF, can nevertheless induce transformation when provided with either of these two factors. Importantly, TGFβ is a normal cellular product and the finding of high quantities in blood platelets and its release during blood coagulation established a clear link with PDGF (Childs et al., 1982).

In screening the transforming effect of TGFβ in numerous tumors, there was an unexpected finding. Depending on the cells and the conditions, TGFβ can either promote or suppress cell growth and transformation. It cooperates with TGFα and EGF to cause cell transformation. On the other hand, it inhibits colony formation in cells derived from human tumors. It appears that its effects are a function of the total set of growth factors and their receptors that are operational at a given time (Roberts et al., 1985). In addition, TGFβ plays a number of key roles in the process of tissue remodeling and wound healing (Sporn et al., 1987). It induces the production of fibronectin and collagen and thus regulates the deposition of the cell matrix, itself a key determinant of cell growth (see Chapter 17).

**PROBLEMS WITH NOMENCLATURE**

As must be evident, nomenclature in this area is arbitrary, to say the least. Some growth factors were named after the cells from which they were first isolated, others from the cells which they stimulated, yet others from the principal action that they appeared to perform (Roberts et al., 1980). In immunology, we hear of interleukins and colony-stimulating factors. These direct the maturation and proliferation of white blood cells. In virology, we have the interferons that “interfere” with viral infection and in cancer research we have tumor necrosis factor (and its relatives) that can influence the growth of solid tumors. In each discipline, it seemed that these factors functioned mainly in the category in which they first came to light. Of course, we now know that some growth factors have actions that are totally unrelated to growth. For instance, PDGF, released from platelets at sites of tissue damage (Kaplan et al., 1979), not only supports the growth of fibroblasts, smooth muscle cells, and glial cells, but also acts to regulate the distribution and migration of vascular smooth muscle cells and fibroblasts in wound healing (Grotendorst et al., 1982).

To add further complexity to an already complex situation, the conditions in which the cells are studied can determine the cellular response, for instance, the presence of other factors, other cells, attachment to substrates. A good example of this is TGF-β. As its name implies, this
emerged as a factor enhancing cell transformation, but we now recognize that it can inhibit cell proliferation (Cone et al., 1988) and that it is a very potent chemotactic factor for neutrophils (Haines et al., 1993) and fibroblasts (Postlethwaite et al., 1987). It has been proposed that a common name for these factors should be cytokines (Nathan and Sporn, 1991). While offering no clues to their various actions, this definition represents a move toward coordinating our understanding of their roles as first messengers. The unity of the cytokines is a concept as important as that of the hormones, defined by Bayliss and Starling nearly 90 years ago.

**Cytokines:** Soluble (glyco)proteins, non-immunoglobulin in nature, released by living cells of the host, acting nonenzymatically in picomolar to nanomolar concentrations to regulate host cell function.

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