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Jean Brachet's cytochemical embryology : connections with the revovation of Biology in France?

Introduction

This paper raises some questions about the possible influence of Jean Brachet (1909-1988) and his collaborators on the formation of molecular biology and the renovation of cytology and biochemical studies bearing on differentiation and embryological development in France in the period from the mid- 1930s until the 1960s. These questions rest on the suspicion that Brachet had a greater influence than one might initially suspect on those responsible for the founding of molecular biology in France, particularly on their formulation of questions and experiments. The question of Brachet's influence in France is a new one, at least for me; as will be clear, this paper is speculative and intended to interest others in pursuing a series of questions only a few of which I will be able to tackle in the next few years. Given that I have little direct evidence to offer at the moment, the paper will consist of two major parts. In the first, I will set background in order to show that my questions about Brachet's influence are well motivated and sensible. In the second, I will provide a moderately detailed account of part of Brachet's work and that of his immediate colleagues; it will be clear from that description that certain of the questions, innovations, and positions we owe to Brachet are concordant with some themes and techniques that characterize the renovation of biology in France during the period in question. In a brief concluding section, I will attempt to integrate the considerations of the two main parts of the paper.

Background and Framework

Let me start with some background about Jean Brachet's contacts with France. His father, Albert (1869-1930), a major embryologist, was well connected in France.[1] When the First World War broke out, Brachet pere was working at the "Laboratoire Maritime" in Roscoff. Unable to return to Belgium, he and his family went to Paris, where he taught during the war as "professeur adjoint" at the Faculte de Medicine. Jean Brachet's contacts with Felix Henneguy and the latter's son-in-law Emmanuel Fauré-Frémiet may date from these childhood days; in any case, Henneguy invited the senior Brachet to give the "Conferences Michonis" at the College de France, lectures that formed the basis of Albert Brachet's book L'Oeuf et les facteurs de l'Ontogenese. Albert Brachet's contributions to French science were honored by his election to the status of "Correspondant" of the Institut de France and by the award of a "Docteur honoris causa" by the University of Paris in 1919.

When Jean Brachet began his studies, to avoid his father's tutelage he worked with his father's assistant, Albert Dalcq (1893-1973).[2] Like the senior Brachet, Dalcq spent his

summers in Roscoff, as did Jean Brachet in the 1930s. There he was in intimate contact with, among others, Boris Ephrussi (Faure-Fremiet's pupil), Andre Lwoff, Jacques Monod, and Louis Rapkine. I shall be explicit about one small aspect of this contact later.

These personal contacts belong to a long series of interchanges, begun by Edouard Van Beneden, with various workers in France. For example, Eugène Wollman, whose important career at the Institut Pasteur is familiar to this audience, was sent by van Beneden to work with Elie Metchnikoff at the Institut Pasteur. It was in honor of Metchnikoff that Elie Wollman received the name 'Elie'. Similar interchanges were continued by the major figures in van Beneden's lineage, i.e., by Albert Brachet, Albert Dalcq, and Jean Brachet. It is my impression that in some parts of embryology and cytology, these contacts managed to overcome the otherwise surprising intellectual distance between French and Belgian science.[3]

Whatever the status of scientific communication between France and Belgium in general, the long lineage of connections just indicated makes it probable that Jean Brachet's work on topics concordant with the interests of French biologists would receive serious attention. This impression is reinforced by the number of interchanges in later years between the laboratories of Brachet and his colleagues and various laboratories in France. I have not had opportunity to investigate these systematically, but will mention a few in passing below.

Given this background, my limited discussion of Brachet's work will establish certain affinities between his interests and those of French workers and will show it to be plausible that his original contributions had some influence on the renovation of French biology. I shall emphasize especially the importance of some physiological aspects of Brachet's embryology, cellular biology, and cytochemistry, work which was important in the study of nucleic acids, nucleo-cytoplasmic relations, and the development of regulatory thinking in molecular biology. Brachet, I shall argue, provided crucial interconnections between studies of the cellular location of molecules and their form and function on the one hand, and studies of determination, embryonic induction and cellular and organismic form and function on the other hand. My questions go to the issue whether his work along these diverse lines was of importance to developments in France.

Before turning to Brachet, it will help to recall some ofthe work he would have encountered at Roscoff. I will treat this work as emblematic of the mixture of continuity and renovation in at least some corners of French biology in the thirties; the value of my questions about Brachet's influence will depend in part on the merit of using the work at Roscoff as a touchstone.

At Roscoff there were many beautiful, though conservative, projects in traditional descriptive morphology and embryology. In addition, there was work on the metabolic and developmental roles of sulfhydril compounds, a topic pursued by Faure-Fremiet and, especially, Rapkine (Rapkine 1931, 1938a,b; Rapkine and Trpinac 1939; Rapkine et al. 1939); Brachet himself took up this topic (e.g., Brachet 1938), and he and Rapkine worked jointly on it after World War II, work terminated prematurely by the latter's death (Rapkine and Brachet 1951; see Zallen, 1991 and in press). Similarly, Brachet must have seen Ephrussi's work on the effects of temperature on the early stages of sea urchin development and discussed the latter's work on tissue culture, his Drosophila work with Beadle, and his reasons for believing that the potentials of eggs and of cells were ultimately under genetic control (see Burian et al. 1988, 1991). Yet further, he would have known Chatton and Lwoff's work on ciliate morphology (in which Monod also participated briefly) and their insistence on the genetic continuity and morphogenetic importance of such cellular organelles as kinetosomes.[4]

At least equally important here was the work of Lwoff and his colleagues on growth factors and nutritional competence in bacteria, ciliates, and other microorganisms.[5] In this research, they dissected the biochemical pathways required for the utilization of various carbon sources in microorganisms and characterized specific steps that were blocked in enzymatically or metabolically deficient organisms that depended on a host or a food source to provide a substance for them. They showed that closely related organisms often differ in their ability to carry out a single synthetic or degradative step (or a small series of related steps) required to utilize a particular carbon source; they held that the ability to carry out these steps was controlled by the presence or absence of an enzyme or enzymes. Enzymes, they claimed, exhibited "genetic continuity" -- i.e., they arose from other enzymes by some sort of duplication and template system rather than being manufactured de novo.[6]

Let me make a side comment here about the importance of this work: Physiological, specifically nutritional, work of this sort depends heavily on kinetic studies of growth and metabolism. Physiological work along such lines provided a crucial background for many early studies contributing to what is now called molecular biology. For example, the work of Lindegren, Monod, Spiegelman, and others on so-called enzymatic adaptation, i.e., the competence of cells to switch from one carbon source to another without genetic change, is essentially physiological and kinetic in character. An immense number of the founding figures of molecular biology, including Beadle, Brachet, Ephrussi, Hershey, Lederberg, Lindegren, Luria, Monod, Pontecorvo, Spiegelman, and many others drew on a background in nutritional studies and allied work -- a fact that I believe is not widely appreciated and the importance of which is not widely understood.

I maintain that the mix of studies represented at Roscoff belonged to a seldom recognized physiological tradition bearing on genetic continuity. In France, later analyses of heredity at the molecular level built upon this tradition (see Burian 1990). To put the point anachronistically, both the morphological and the physiological studies were later put to use in the attempt to determine whether a particular feature or physiological capacity (e.g., the activity of a particular enzyme) was under genetic control -- and if so, what combination of nuclear and cytoplasmic controls regulated the formation or behavior of that feature. The embryological notions of determination and differentiation, the non-Mendelian (or, rather, a-Mendelian) notion of genetic continuity, and the tools of kinetics and of localization of events within cells, tissues, and organs were all important to this work. The importance of morphological studies is less obvious than that of physiological ones. As I shall show, Brachet's work, particularly his cytochemistry (which depended on localization of physiological processes in particular organelles or cellular regions) is particularly important in just this respect.

To prepare for the discussion of Brachet's research, let me close this section with a brief comment about biochemistry. The point of interest is this: In the forties and fifties, one could often distinguish (molecular?) biologists from biochemists because of the chemical, rather than biological, orientation of biochemists. Many biochemists treated the chemical formula, isomeric structure, and chemical interactions of a molecule as telling very nearly the whole story, believing that the physical binding of enzymes or proteins to membranes, or the location of enzymes on or in organelles, was, at best, of secondary importance. To be sure, as Claude Debru has reminded me, there were many exceptions to this based on strong traditions like that of Meyerhof, but the image of the biochemistry of the day as treating the cell as a bag of enzymes is not entirely a caricature. It has something to do, for example, with the resistance to Peter Mitchell's chemiosmotic hypothesis. In this respect, many biochemists considered aspects of biological form or structure to be of secondary or tertiary interest. In particular, the ideas that proteins or

enzymes would function differently in different cell compartments and that one had to take account of their interactions with membranes or organelles, commonplace to many biologists, were foreign to most (but by no means all) biochemists. Be this as it may, many biochemists were not particularly interested in localizing particular biochemical interactions on particular structures.[7] Jean Brachet, raised in the tradition of causal embryology and germinal localization (which was one of Dalcq's principal interests), was not, in this respect, a biochemist. Indeed, the relevant parts of his work are better described as cytochemistry than biochemistry, for they are concerned with the cellular location and the local action of the compounds studied by biochemists. With that, we turn to Brachet's research.

Brachet's Chemical Embryology and the Determination of Nucleic. Acid Function.

Jean Brachet's use of cytochemical techniques in the hope of understanding development and its controls led him, as we shall see, to make fundamental contributions to molecular genetics. Brachet's work on chemical embryology began as an extension of Albert Brachet's and Albert Dalcq's causal embryology. Our immediate interest concerns his effort to understand the synthesis, localization, and physiological roles of nucleic acids in embryonic development, which constitute only a small portion of his largely embryological research. As early as 1933, he published results obtained with virgin sea urchin eggs suggesting that "yeast" (or pentose) nucleoproteins were present exclusively in the cytoplasm and that small quantities of "thymus" nucleic acid were present in the nucleus (Brachet 1933). In spite of the anachronism, for convenience I shall use the modern labels RNA and DNA for the two nucleic acids from here on. But it is worth remembering that in 1933 "yeast" nucleic acid was thought to occur only in plants, "thymus" nucleic acid only in animals, and the latter was thought to be a boring tetramer, perhaps buffering cellular pH. In 1933, having found large quantities of RNA in the cytoplasm of sea urchin eggs, Brachet noted that after fertilization the amount of RNA in the embryo's cytoplasm decreased roughly in synchrony with the increase in nuclear DNA. He hypothesized that the pentoses in the cytoplasmic RNA served as a reserve of precursors that were transformed into DNA during embryonic development.

In a valuable retrospective account of this work, Brachet writes revealingly about his second summer of work on this project:

«*I went back to Roscoff in 1932, where I measured the pentose content of sea urchin eggs despite the ironical comments of my French friends (B. Ephrussi, A. Lwoff, J. Monod): at that time, pentoses were believe to exist only in plant and the method I was using has been devised to measure the pentosan content of ... Nevertheless, ... I found that sea urchin eggs and embryos indeed contained large amounts of a pentose derivative, which was later identified as RNA* » (1983, p. 171; see also Brachet 1987).

Under pressure from Dalcq, Brachet devised (and then largelyrelied on) cyto chemical methods to localize RNA and DNA and make them visible. He employed Feulgen, Unna and toluidine blue stains -- the second of which usually stains RNA red and DNA green. These were combined with the use of deoxyribonuclease and (after 1938) ribonuclease to confirm that the color produced was, indeed, due to the presence of the suspected nucleic acid. Over the years, these techniques were refined and cross-checked (as described in Brachet 1947a), and the findings obtained by their use integrated with more sophisticated techniques and hypotheses. The spirit of his enterprise, however, is already clear in the paper of 1933.

By 1942 he had demonstrated high concentrations of RNA in nucleoli and in the ergastoplasm (i.e., basophilic cytoplasm, especially what was soon recognized as endoplasmic reticulum), but also showed that up to 10% of the nucleic acid in nuclei is RNA (Brachet 1942; see also Brachet 1940a,b, 1941; Caspersson 1941). As early as 1933, the high concentration of RNA in cells producing large quantities of protein or enzymes suggested that RNA might somehow be connected with protein synthesis. This suggestion, at first based on rather diffuse evidence, came to play an ever-larger role in Brachet's subsequent discussions of the physiology of nucleic acids.

By 1944 the synthesis, localization and physiological roles of nucleic acids had already become important enough that Brachet devoted 56 of the 500 pages of his Embryologie chimique to it (Chap. VI, pp. 194-250). Like many cytologists of the day, he thought chromomeres might correspond to genes. Although he did not identify genes with nucleic acid, he argued, against Koltzoff (1939), that the proportion of DNA in chromomeres was constant throughout the cell cycle, so that DNA might be a constant component of genes (pp. 70-71). He demonstrated a relationship between the quantity of RNA and the amount of synthetic activity in a cell, noting, inter alia, that an increase of RNA is required for a specialized cell to begin facultative production of its distinctive product and that secretory functions are proportional to the amount of RNA in the endoplasmic reticulum. He showed that RNA in the endoplasmic reticulum is associated with microsomes, as isolated by Albert Claude (1941, 1943) using ultracentrifugation. The smallest microsomes maintain a constant ratio of nucleic acid to protein, though they also form larger units containing more protein and, perhaps, agglomerate to form mitochondria. And there are hints of new findings better articulated in an important paper given in 1948 on "L'hypothese des plasmagenes dans le developpement et la differenciation," at a symposium on Unites biologiques douees de continuite genetique (Brachet 1949, discussed in Burian 1990).

The paper makes some important additions to Brachet's account of nucleic acid physiology. The smallest microsomes, or ribonucleoprotein granules, have a remarkably constant chemical constitution. Their importance in protein synthesis is shown by the fact that in cells making a lot of a particular product, e.g., hemoglobin, granules with varying amounts of the product attached to them can be isolated. The granules exhibit a sort of growth, starting from their basal size:[8] "il se produit dans la cellule une sorte de .croissance/ des granules, consistant dans l'accolement autour d'une sorte de germe nucleoproteique de molecules diverses : proteines douees ou non d'activite enzymatique et lipides" (Brachet 1949, 156). Brachet reports parallel findings for respiratory enzymes and cytoplasme "sont synthetises par le noyau" (p. 157). The most plausible hypothesis, he holds, is an extension of that of Morgan (1934): regional cytoplasmic differences in the distribution of ribonucleoprotein particles irreversibly alter the chemical activity or constitution of nuclei in such a way that they produce distinctive (self perpetuating?) ribonucleoprotein particles, thus committing different cells and cell lineages to distinct differentiated functions.

The resultant picture is consistent with, but does not decisively favor, an account of biosynthesis that treats the ribonucleic granules as plasmagenes. Such an account would involve nuclear synthesis (or nuclear regulation or activation) of cytoplasmic granules with specific competences. If the granules were self-reproducing, which is precisely what was meant by "douee de continuite genetique," and if they, in turn, synthesized (or controlled the synthesis or specificity of) proteins and other cellular products, one would have the outline of a general solution to the problem of protein synthesis. A huge number of details were missing -- and, in their absence, Brachet was duly skeptical of the

plasmagene theory -- but at least the picture was consistent with the available information and provided a clear direction for further research.

The fact that Brachet presented his work at this symposium already suggests that he played an important role in the post-war renovation of French biology. This meeting, afte all, was meant to set the direction of post-war genetics in France (Zallen 1989). And unlike the other foreign participants, Brachet remained in continuous contact with his French colleagues. Furthermore, the paper's emphasis on the plasmagene hypothesis is entirely concordant with the directions taken by Boivin, Ephrussi, l'Heritier, Lwoff, Monod, Rizet, and others during the late forties and early fifties (Burian 1990).

By 1950, Brachet recognized a complication for the plasmagene hypothesis. He found that at least one more fraction of RNA, remaining in the supernatant after ultracentrifugation, is required by cells that actively produce some protein or are ready for major growth. This fraction was somehow connected with the specificity of the syntheses performed by the cell. There are some complications yet to be resolved in understanding the historical sequence of Brachet's treatment of this RNA because of its relation to so-called "soluble RNA." In American usage by the mid-fifties, "soluble RNA" was produced at pH 5; this picked out what was later known as transfer RNA. I have yet to puzzle out Brachet's usage around 1950, but judging by the quantities he measured, there were probably transfer RNAs, non-membrane-bound polysomal RNAs, and perhaps some messenger RNAs among the RNAs remaining in the supernatant (though Brachet did not recognize the distinctions among them), for he determined that "a large portion of &]RNA~, which may exceed 50 per cent of the nucleic acid present in the extracts of frog eggs and embryos, as well as of chick embryos, is not sedimented by ultracentrifugation" (Brachet, 1950, 864).[9]

By 1952, Brachet produced a 122 page review, "Le role des acides nucleique dans la vie de la cellule et de l'embryon," published by both Masson and Desoer. I am not sure how influential it was, but it provides a remarkable capsule of the state of knowledge at the time and shows very clearly that the mixture of localization, cytochemistry, and kinetics had already produced an early version of what, thanks to Francis Crick, later came to be known as "the central dogma" of molecular biology. ** Show diagram. ** The scheme is a dynamic one, with at least some feedback loops (and room for more), in which nuclear DNA makes RNA that associates with microsomes in the cytoplasm (including what were later called ribosomes) to make cytoplasmic proteins. These processes depended on ATP produced in the mitochondria which, themselves, might be the product of agglomerations of microsomes. This diagram illustrates a quite different pathway into molecular genetics and the central dogma than that of Crick or of most American workers, a pathway not untypical of that of many French biologists. It is not unreasonable to suggest that the work that led up to it, intimately known by those French workers, was of importance in shaping the antecedents of French molecular biology in the thirties and forties.

Let us return briefly to the supernatant RNA. Although the supernatant contained an unholy mess of different RNAs, over the next few years Brachet and others were able to separate them into distinct classes and to show that they are needed to determine protein specificity. Furthermore, he already knew that ribonuclease stops protein synthesis. Sometime before 1960 (I have yet to determine the precise date), he also knew that addition of ribosomes to a cell stimulates protein synthesis, but non-specifically. That is, he knew that the specific protein produced depends on some RNA in the host cell other than ribosomal RNA, and not on the source from which the injected ribosomes were derived.[10] Once this was clear, it was obvious that for RNA to be the intermediary between the genes and the production of protein, the supernatant RNA or some other

RNA fraction would have to be responsible for the specificity of protein synthesis. It is important to fill in the case study properly, for this is part of the background to Jacob and Monod's work on messenger RNA. For this reason, it will be crucial to determine precisely when Brachet established these results and the extent to which they were known and accepted.

One special investigation deserves particular mention, for it provides an elegant illustration of the concordance of Brachet's concerns with, and the differences of his techniques from, those of Ephrussi, Jacob, Lwoff, Monod, and their colleagues. The studies in question concerned the extraordinary giant unicellular alga, Acetabularia mediterranea. This organism, though composed of a single cell, grows to over two centimeters in length, with a long thin stalk and a foot (rhizoid) in which the nucleus is located. When it is ready to reproduce, it forms an umbrella-like cap in which its spores are located. The most striking finding about Acetabularia, already reported in Hämmerling (1934), is that it can live virtually indefinitely -- for a period of many months -- after its nucleus is excised. Even more striking, and the subject of a large number of studies by Brachet and his colleagues (reviewed in Chantrenne, 1961), is the fact that for at least two months after its nucleus has been excised, a capless individual can still regenerate an umbrella. Thus, Acetabularia can carry out a major morphogenetic step in the absence of a nucleus. Nonetheless, if an enucleate fragment is supplied with the nucleus of a related species, the cap assumes the morphology of the donor of the nucleus rather than that of the recipient. It follows that the genetic determinants of the umbrella are provided by the nucleus, but stored for a long period in the giant cell.

Since Brachet had available the techniques to test whether RNA and DNA are manufactured by enucleated Acetabularia, this organism played an important role in working out nucleo- cytoplasmic relations, the extent of nuclear control of morphogenesis, and the role of the nucleus and other organelles in the manufacture of nucleic acids. Suffice to say that by 1951 he was able to demonstrate that protein synthesis continued months after the enucleation of the cell (Brachet and Chantrenne, 1951), but that over time the proteins produced were less frequently nuclear in origin and more frequently chloroplastic.[11]

Thus, by 1951 Brachet and his colleagues knew that cap formation in enucleated Acetabularia involved genuine protein synthesis under nuclear control. For a certain period of time, the enucleate fragments of the alga were thus able to utilize genetic information, derived from the nucleus but stored in the cytoplasm, to make protein. By 1960, Brachet was able to distinguish fairly cleanly between RNAs produced by the nucleus and those produced by chloroplasts and mitochondria. This enabled him to argue that "cytoplasmic ribonucleic acid carries the genetic information originating from the genes, and controls, for a certain time in any event, synthesis of specific protein" (Brachet 1960b, 197) and that "a true synthesis of chloroplastic ribonucleic acid occurs in ... anucleate Acetabularia; this synthesis takes place at the expense of the other cytoplasmic ribonucleic acid fractions" (ibidem).

Conclusion

The portion of Brachet's work examined here centered on the physiology of the nucleic acids, their role in protein synthesis, and the differences in their distribution and behavior in different cellular compartments. Relative to prior knowledge in embryology, biochemistry, and genetics, his findings were novel. Yet, his larger purpose was traditional: to understand morphogenesis and the control of development and

differentiation. This combination of novel techniques and findings applied to traditional questions speaks to the theme of this symposium -- the inextricable mixture of continuity and renovation. About this dialectic I will make only one comment: in France and Belgium the protection offered by the system of professorial appointments and laboratory headships at least occasionally allows an investigator to pursue difficult questions, not readily accessible to empirical resolution, for an entire career. This happens less readily in the United States, where funding for research and evaluations of personnel are usually short term. This difference, less important in this day of big science than it used to be, plays some role in the continuities that, for better or worse, are visible in French biology.

Jean Brachet was well connected with French biologists during the period to which this symposium is devoted (and continued to be so in subsequent years; see Gaudilliere 1991). His research showed strong affinities to (as well as some differences from) the research of the major figures who transformed French biology during this period. I have been able only to hint at the similarities and differences, though I would stress particularly the similarities in the larger questions that motivated his, and their, research. Yet Brachet stands far enough outside the French scene to allow us to ask, by comparing and contrasting his work with that of analogous figures in France (perhaps especially Boris Ephrussi and Etienne Wolff), what is distinctively French during this time in the disciplines in question. Alas, I am not yet able to produce such comparisons in detail, but I hope I have shown that they are likely to be worthwhile.

It is not clear whether the affinities between Brachet and various French biologists are mainly the effect of mutual influence during periods of contact or whether other causes played an important role. Among the common influences that must be taken into account are the shaping role of the disciplines and traditions in question (e.g., causal embryology, cytology, nutritional biochemistry, genetics, Pasteurian microbiology) on the questions investigators pursued -- e.g., about the control of differentiation, the localization of certain compounds or events, or the regulation of cellular states. Similarly, one must ask whether the effect of commitment to certain techniques (e.g., biochemical kinetics) to answer standing questions can explain the similarities of approach and of stance suggested by my exposition. Finally, one should ask whether there is a distinctive French style (Burian and Gayon, in progress) or whether local cultures have a greater effect than national style on the substance of biological research. Only a series of fully comparative studies, in the spirit of Gaudilliere's work comparing Monod and Spiegelman (Gaudilliere in press), can get to the root of these issues.

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Notes

[1] All of the claims about Albert Brachet's French connections made here are drawn from Dalcq (1968).

[2] For an account of Dalcq's career, see Pasteels 1980.

[3] This suggests a speculative question: does this background have anything to do with the relative sympathy of Eugene Wollman for the views of Jules Bordet and Andre Gratia, as compared with those of Felix d'Herelle, about the nature of bacteriophage?

[4] See e.g., Chatton and Lwoff, 1929; Chatton et al. 1931. For a brief account of this work and for references, see Burian and Gayon, 1991.

[5] This work was not done at Roscoff, but presumably would have been discussed there. The early findings are summarized in Lwoff,(1932); later parts of it are presented in Lwoff (1943); cf. the discussion in Burian and Gayon (1991).

[6] The terminology of genetic continuity may mislead the contemporary reader. Here it means that the enzyme had to be produced from preexisting enzyme or a generalized precursor of the same nature as enzymes. In general, structures counted as genetically continuous if, like cells, chromosomes, and nuclei, they could arise only out of preexisting structures of the same sort. By the 1940s, there were many candidates for this status: chloroplasts, chromosomes, enzymes, genes, kinetosomes, mitochondria, nuclei, nucleoli, plasmagenes, viruses, and many more. Some workers held that biology should be built on the study of genetically continuous entities since organisms, themselves genetically continuous, are constructed, perhaps indirectly but primarily, by combination of parts that are genetically continuous. Lwoff employed this terminology in reference to organelles (specifically kinetosomes) as early as 1929 and soon after to enzymes; cf. Chatton and Lwoff (1929).

[7] Piotr Slonimski has stressed this point in interviews, suggesting that this (and failure to adopt a genetic approach) was a major barrier to the analysis of mitochondria in the late fifties and early sixties.

[8] These results are amplified by Brachet's collaborator, H. Chantrenne (Chantrenne 1947) and discussed in Brachet (1947a), n.

[9] Brachet (1950). The centrifugation was at 100,000 G for less than an hour. An initial report of this finding in frog eggs is given at p. 24 of Brachet, (1947b).

[10] Brachet (1960a); these results are taken from Chap. 1, Sect. 4, "The role of the microsomes and ribonucleoprotein particles in protein synthesis."

[11] The evidence for this last claim was only suggestive until sometime after 1963, when CsCl density centrifugation of the DNAs produced in enucleate Acetabularia confirmed beyond question the source of the RNAs produced at different times after enucleation. Cf. Green et al., (1967).