

EXPLORATORY EXPERIMENTATION AND THE ROLE OF HISTOCHEMICAL TECHNIQUES IN THE WORK OF JEAN BRACHET, 1938-1952

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EPIGRAPHS

[There are serious limitations on the available histochemical, biochemical, and physical methods for estimating precise quantities and locations of nucleic acids.] Reliable results can therefore only be obtained if all available tests are applied and give concordant results; it is also imperative, whenever possible, to check cytochemical results with microchemical estimations of pentose- and thymo-nucleic acids. For instance, thymonucleodepolymerase has no effect on the basophily of some spermatozoa and red blood cells; it would be a mistake to conclude that these nuclei are devoid of thymonucleic acid, since they give a strong Feulgen test; microchemical estimations show that they are rich in the said acid: the correct conclusion is that the thymonucleic acid is not attacked by thymonucleodepolymerase in these nuclei; this is an interesting fact in itself, which deserves further studies on the nature of the thymonucleic acid present in these cells and on the links uniting this acid to the proteins. The value of microchemical analytical methods must certainly not be forgotten, even if these techniques are incapable of the same refinement as the cytochemical ones.¹

Clearly, much work remains to be done along these and other lines; but it is an encouraging fact that progress is being made towards our ultimate goal: the establishment of closer links between such different sciences as cytology, embryology, biochemistry and genetics.²

[As the history of cell physiology and biochemical cytology in recent years shows,] overspecialization is to be carefully avoided. The biologist who is interested in cell physiology should not be a morphologist, or a physiologist, or a biochemist: he should not only be capable of using physiological and biochemical methods as well as the microscope, but he should utilize them all in attacking his problem. Neither the variety of the methods nor the acquisition of a wide knowledge in very different fields should frighten him. This is the price which has to be paid if cell physiology is to progress. The same price has been paid in the field of biochemical genetics of micro-organisms, in which such outstanding advances have been made recently.³

¹ J. Brachet, 'Nucleic acids in the cell and the embryo', *Symposia of the Society for Experimental Biology*, 1 (1947): 207-224, quotation from pp. 208-209.

² *Ibid*, 222.

³ J. Brachet, *Biochemical Cytology*, New York: Academic Press, 1957: 3-4.

INTRODUCTION

As a young medical student in the late 1920s, Jean Brachet was asked to choose between two problems for his first laboratory project — the production and role of so-called mitogenetic rays in developing embryos and the localization of thymonucleic acid, now known as DNA,⁴ in growing oocytes.⁵ Fortunately for him and for many others, he chose the latter project. Not long afterward he extended his project to include pentosenucleic acids (now known as RNAs) as well as thymonucleic acids. In a sense, the localization and physiological functions of nucleic acids remained one of Brachet's central research interests throughout his long career. The problems surrounding the localization and functions of nucleic acids served as a common technical entry into nucleo-cytoplasmic relations, embryogenesis, chemical embryology⁶ and biochemistry of development, and molecular genetics while allowing him to remain faithful to embryological and developmental issues that could not be addressed or resolved by use of procaryotes. His early experiments bearing on this topic were carried out in a style of exploratory experimentation that *should* be of great historical and philosophical interest, but to which philosophers of science have not paid much serious attention. One important exception is Hans-Jörg Rheinberger, who has been developing an epistemology of experiment based explicitly on exploratory work of the character that is at the center of interest in this paper.⁷ As the careers of Brachet and many of his

⁴ It is worth paying a bit of attention to Brachet's terminology for nucleic acids. In his earliest papers, Brachet used the terms *acide thymonucléique* or *thymonucleic acid* and *acide pentosenucléique* (of which the best known example was *acide zymonucléique*) or *pentosenucleic* and *zymonucleic acid* from yeast. This terminology reflects the view, quite widespread in the 1920s and 1930s, that DNA is an animal nucleic acid, particularly prevalent in the thymus, and that RNA is a plant nucleic acid, easily obtained from yeast, although it was also known to be present for unknown reasons in the pancreas of certain animals. I do not believe that Brachet was committed to these views in his early papers. At p. 525 of J. Brachet, 'Recherches sur la synthèse de l'acide thymonucléique pendant le développement de l'œuf d'Oursin,' *Archives de Biologie* 44 (1933), 519-576, he speaks of the presence in animal tissues of "acides ribonucléiques (pentosenucléiques) très voisins de l'acide nucléique de la levure" and knows that the "sucre constitutif de l'acide thymonucléique" is "d-ribodésosé" (525-526). By the late 1930s, he regularly used the term *acide pentosenucléique* for RNA; by 1944 (J. Brachet 'Acides nucléiques et morphogénèse au cours de la parthénogénèse, la polyspermie, et l'hybridation chez les anoures,' *Annales de la Société royale Zoologique de Belgique* 75 (1944): 49-74) this was replaced by the terms *acide ribonucléique* and *ribonucleic acid*. He started using the term *deoxyribonucleic acid* in English regularly in 1947 (J. Brachet, 'The metabolism of nucleic acids during embryonic development', *Cold Spring Harbor Symposia in Quantitative Biology* 12 (1947), 18-27), but apparently did not adopt its French equivalent, *acide desoxyribonucléique* regularly until about 1950. I will not follow his terminology rigidly, partly because he was clear early on about the specific differences between the sugars in RNA and DNA and knew that RNA, at least, was no more a plant than an animal nucleic acid. [I have not noticed when he was specifically aware of DNA in plant nuclei.] It should be kept in mind, however, that these shifts in terminology may mark some differences in background beliefs.

⁵ J. Brachet 1957 (footnote 3), p. 1; see also 'From Chemical to Molecular Sea Urchin Embryology', *American Zoologist* 15 (1975), 485-491, p. 485.

⁶ A useful source for some of the work discussed here is B. Fantini *Histoire de l'Embryologie Chimique*. Dissertation, Paris: Université de Paris I, 1978.

⁷ In addition to a long series of papers [e.g., H.-J. Rheinberger, 'Experiment, Difference, and Writing. I. Tracing Protein Synthesis. II. The Laboratory Production of Transfer RNA', *Studies in the History and Philosophy of Science* 23 (1992), 305-331 and 389-442; *Experiment, Differenz, Schrift: Zur Geschichte epistemischer Dinge*, Marburg: Basiliskenspre, 1992; and 'Experiment and Orientation: Early Systems of *in vitro* Protein Synthesis', *Journal of the History of Biology* 26 (1993), 443-471], see

colleagues show, such exploratory work is a high art that can sustain long-term and productive research programs. In this paper, I will examine some of Brachet's early work on localization of nucleic acids and parts of the pathway (other parts of which are explored in Rheinberger's contribution to this issue) by means of which that problem led him, Hubert Chantrenne, and Raymond Jeener to be concerned with the problem of protein synthesis. I will employ these materials to support some concluding remarks about exploratory experimentation.

LOCALIZATION OF PENTOSENUCLEOTIDES AND THE SYNTHESIS OF THYMONUCLEIC ACID

In an important early paper on 'his' topic,⁸ Brachet sets forth one of the central problems of his early research on localization and metabolism of thymonucleic acid. The problem starts from a controversy between Jacques Loeb and others on the one hand⁹ and Godlewski and others.¹⁰ Loeb and Godlewski both found very little nucleic acid in the nucleus of virgin sea urchin eggs (as well as other eggs). They also agreed that the net quantity of thymonucleic acid in the nuclei of the cells of the blastula and the gastrula increases considerably in the course of normal development and that the quantity of thymonucleic acid continues to increase at least until the pluteus is formed. But Loeb held that the nucleic acid is formed by an autocatalytic process. While we currently think of this in terms of *de novo* synthesis, Loeb's conception was of autocatalysis was different. It was compatible, for example, with a template impression process of some sort on pre-existent materials, and not particularly tied to the idea of construction of a molecule from relatively simple raw materials in the cytoplasm. Godlewski, in contrast, held that the germinative vesicle of the immature oocyte contains a reserve of nucleic acid that diffuses into the cytoplasm of the maturing egg when the vesicle bursts. Thus Godlewski held that the nucleic acid was not formed autocatalytically, but migrated from the cytoplasm into the newly forming nuclei. Since the volume of the germinative vesicle is roughly equal to that of the sum of the volumes of the nuclei of the young pluteus and, since there was no way to measure the quantity of nucleic acid directly at that time, the issue was unresolved when Brachet began to work on it in the late twenties.

In his 1933 paper, Brachet developed and supported a tentative conclusion that he had already reached by 1931 and which, if true, helped to resolve the contradiction between Godlewski and Loeb. His evidence was based on Fuelgen staining, which responds to DNA but not RNA, and on indirect measurements of the total quantities of pentose nucleotides,

particularly *Experimental Systems. Towards a History of Protein Synthesis. Synthesizing Proteins in the Test Tube*, Stanford: Stanford University Press, in press.

⁸ See J. Brachet, 1933 (footnote 4); also 'Recherches sur le comportement de l'acide thymonucléique au cours de l'oogénèse chez les diverses espèces animales', *Archives de Biologie* 39 (1929), 677-697; 'L'évolution des pentoses pendant le développement de l'œuf d'Oursin', *Comptes rendus de la Société de Biologie* 108 (1931), 1167-1169; and *Embryologie chimique*, Liege and Paris: Desoer and Masson, 1944, which reviews all of the work up to that date. See esp. chap. VI, "Synthèse, localisation et rôle physiologique des acides nucléiques," pp. 194-250.

⁹ See, e.g., J. Loeb, *University of California Publications in Physiology* 3 (1907), 61 ff. and 'La fécondation chimique', *Mercure de France* (1911).

¹⁰ See, e.g., E. Godlewski, 'Plasma und Kernsubstanz in der normalen und der durch äussere Faktoren veränderten Entwicklung der Echiniden', *Archiv für Entwicklungsmechanik* 26 (1908), 278-328 and especially 'Eireifungsprozess im Lichte der Untersuchung der Kernplasmarelation bei Echinodermkeimen', *Archiv für Entwicklungsmechanik* 44 (1918), 499-529.

constituents of RNA, in the cell.¹¹ The latter measurements revealed the presence of zymonucleic acid in egg cytoplasm. The finding was controversial since, as the very name indicates, RNA was then thought to be primarily a plant nucleic acid. Brachet sided with Loeb, arguing that there is increase in thymonucleic acid since the egg contains very little of this substance and the total quantity in the embryo increases dramatically in synchrony with the formation of new nuclei. But, for sea urchins and amphibia, Godlewski was right that DNA is not synthesized autocatalytically. Rather, it is produced by transformation of a precursor — specifically the pentose nucleotides of the RNA with which the egg is richly stocked and which provide the material from which DNA is formed in early ontogeny.¹²

Brachet thus argued that sea urchins and amphibian embryos exhibit ‘partial synthesis’ of thymonucleic acid, in contrast to the embryos of chickens and mammals, which did not have detectable reserves of pentose nucleotides and thus exhibited total synthesis, as Loeb had claimed.¹³ The evidence for partial synthesis is complex, but the key point is the exact parallel between the measured decrease of pentose nucleotides in the cytoplasm of the embryo and the increase in nuclear DNA. Brachet remained convinced of the correctness of this account of partial synthesis, and accumulated evidence of increasing strength to support it, until the late 1940s.

STAINS AND RIBONUCLEASE

The effort to arbitrate between Godlewski and Loeb demonstrated the inadequacy of the available techniques to resolve many issues regarding localization and synthesis of DNA and RNA.¹⁴ Brachet was thus forced to develop tools for investigating nucleic acid synthesis and degradation. In the process, by noting specific effects of nucleic acids and their byproducts on young embryos, he found strong connections between his work and work on embryonic induction and the biochemistry of the organizer by Dorothy and Joseph Needham and C. H. Waddington. This led him to investigate, partly in collaboration with these authors, the

¹¹ These measurements were based on Masing’s and the Needhams’ methods for measuring total cellular phosphorus in nucleotides and nitrogen in purines. They required prolonged hydrolysis of the material and directly measured the production of furfural. See J. Needham and D. M. Needham, ‘Phosphorus Metabolism in Embryonic Life. I. Invertebrate Eggs’, *Journal of Experimental Biology* 7 (1930), 317-347 or Brachet’s descriptions of these methods in J. Brachet 1933 (footnote 4), p. 534 and J. Brachet, ‘La détection histochimique et le microdosage des acides pentosenucléiques (tissus animaux -- développement embryonnaire des amphibiens)’, *Enzymologia* 10 (1941), 87-96: 88 ff.

¹² Brachet 1933 (footnote 4), 539-541.

¹³ Brachet contrasted the eggs of amphibia, sea urchins, and numerous marine organisms with those of chickens and mammals. He obtained no evidence of an RNA reserve in the latter eggs, but found simultaneous synthesis of DNA and RNA shortly after fertilization. For many years he separated eggs into two classes according to their presumptive mechanism for manufacture of embryonic DNA — those that synthesized DNA more-or-less totally and those that transformed pre-existing materials to produce DNA. By the mid-1940s, he no longer considered this division to be fundamental since he then thought that embryonic synthesis of RNA from other materials was part of the mechanism for protein synthesis and that it was consistent with consumption of RNA in order to produce DNA. The two classes of eggs thus reflected the time at which protein synthesis began, not differences in the mechanisms for biosynthesis of DNA.

¹⁴ See also the grounds for scepticism about the techniques raised by others, e.g., E. Le Breton. and G. Schaeffer, *Variations biochimiques du rapport nucléoplasmique au cours du développement embryonnaire*, Paris: Masson, 1923.

possibility that pentose nucleic acids might be an inducer or even the primary inducing substance.¹⁵ By the early 1940s Brachet added another problem domain in a somewhat similar manner to the topics he was exploring, namely, the problem of the role of RNAs in protein synthesis.¹⁶ Thus the tools he devised, appropriated, and adapted, were put to different uses in the service of a variety of problems, explored in parallel. In all of these studies, he sought to employ a wide range of organisms and the widest possible range of techniques in order to provide a basis for reconciling the findings obtained and for achieving broad understanding of the process in question. Throughout his career, his attitude appears to have been consistent with the views expressed in the epigraphs to this talk.

I turn to the techniques that Brachet employed in the late 1930s and early 1940s. In his big paper of 1933, Brachet pushed the available methods very hard. Working with sea urchins, he confirmed that the Feulgen stain does not respond to lipids, that it measures thymonucleic acid, that virgin eggs yield almost no Feulgen stain,¹⁷ that there is no measurable thymonucleic acid in virgin eggs by other tests, that a large variety of measures show that there is a considerable reserve of pentosenucleic acids in the cytoplasm, and that the best available technique for estimating total pentosenucleic acid (a rather destructive measurement of the amount of furfural produced in a particular protocol requiring extensive high temperature hydrolysis in highly acid conditions)¹⁸ shows that the time course of RNA in the cytoplasm is,

¹⁵ See e.g., C. H. Waddington, J. Needham, and J. Brachet, 'Studies on the nature of the amphibian organization center. III. The activation of the evocator', *Proceedings of the Royal Society, B* 120 (1936): 173-198.

¹⁶ Rheinberger's contribution to this symposium contains important additional insights into Brachet's early conception of the problem of protein synthesis and the pathways by which he came to this problem. A more thorough exploration of the way this problem took shape should include an effort to understand at exactly what point the problem of protein synthesis was recognized *as such* as an experimental problem by Brachet and his colleagues and by others with whom they were in touch. This issue is crucial for identifying and understanding what Rheinberger, in a different context, has called the 'epistemic objects' at stake. If one is to understand what it is that a prepared mind can discover, one must have some notion of the epistemic objects the investigator is prepared to encounter. See the Rheinberger references in footnote 7 and 'From Experimental Systems to Cultures of Experimentation'. In: G. Wolters and J. Lennox, in collaboration with P. McLaughlin (eds.), *Concepts, Theories, and Rationality in the Biological Sciences: The Second Pittsburgh - Konstanz Colloquium in the Philosophy of Science*. Konstanz and Pittsburgh: UKV-Universitätsverlag Konstanz and University of Pittsburgh Press, 1993: 107-122 for treatment of the epistemic objects that different investigators dealt with in research concerning protein synthesis and the intermediates between the genetic material and the production of protein). Similarly, the persistence of one description or *problematique*, or its exchange for another, is crucial to understanding what Jean-Paul Gaudillière and I, among others, have called the 'local cultures' of particular laboratories. See, e.g., R. Burian, 'Technique, Task Definition, and the Transition from Genetics to Molecular Genetics: Aspects of the Work on Protein Synthesis in the Laboratories of J. Monod and P. Zamecnik', *Journal of the History of Biology* 26 (1993): 387-407 and J.-P. Gaudillière, 'Molecular Biology in the French Tradition? Redefining Local Traditions and Disciplinary Patterns,' *Journal of the History of Biology* 26 (1993): 473-498.

¹⁷ Importantly, *almost none* is not the same as none. R. Thomas (pers. commun.) stresses the importance of Brachet's insistence already at some point in the 1930s of the continuous presence of DNA throughout the cell cycle. I have not located a clear citation demonstrating this. However, in Brachet 1944 (footnote 8), 70-71, he argues against N. Koltzoff (e.g., *Les molécules héréditaires*, Paris: Hermann 1939) that the constant proportion of DNA in chromomeres throughout the cell cycle meant that DNA could be a constant component of genes. This rests also on Brachet's earlier findings of the continuous presence of DNA in the very material (*Triton*) studied by Koltzoff. However, Brachet does not provide a specific citation to an earlier publication stating these results.

¹⁸ "Nous ne possédons malheureusement pas de méthode histochimique permettant de déceler sur coupes les pentosenucléoprotéides : les réactions colorées caractéristiques de ces sucres sont en général

within the error of measurement, exactly the inverse of that of the production of DNA. Such a methodology is, of course, not specific enough to show that the cytoplasmic RNA serves as a precursor for DNA, and Brachet specifically indicates the need for a technique that would allow the histochemical detection of RNA in thin sections.

Somewhere around 1938 he found that method.¹⁹ It involved the use of the so-called 'Unna stain' — a stain employing pyronine and methyl green — together with the use of ribonuclease in alternate thin sections. Ribonuclease was newly available in recrystallized form as well as in cruder preparations. Brachet's protocols had a number of virtues. The two stains in the Unna formula tended to stain RNA and DNA differentially (RNA red, DNA green). The use of ribonuclease allowed double checking whether stained material was really RNA or not, because when the enzyme (which was highly specific and rapid in its action) depolymerized RNA, it removed the color only where RNA was present. Furthermore, comparison with parallel Fuelgen staining allowed cross-checking of the proportion of staining that was genuinely due to DNA.²⁰ The result was a considerable increase in spatial and temporal sensitivity — and strong support for the working hypothesis of partial synthesis.

MULTIPLE PURPOSES, MULTIPLE PROJECTS

Brachet's purposes were multiple and his commitments were sometimes very strong — as is illustrated by the fact that he held on to his account of partial synthesis of DNA for at least fifteen years, building up an impressive stock of evidence in its favor before becoming convinced that the production of furfural did not adequately measure the quantity of RNA present in a cell and that the RNA in virgin eggs had another function.²¹ But as he appropriated

basées sur la formation du furfural qui ne peut être libéré qu'à la suite d'une hydrolyse prolongée, à haute température et en milieu fortement acide ; les préparations histologique ne résistent naturellement pas à pareil traitement." (J. Brachet 1933 (footnote 4), 554). ["Unfortunately, we do not have histochemical methods that permit the detection of pentosenucleoproteins in thin sections. The characteristic reactions of these sugars with coloring agents are based, in general, on the formation of furfural, which cannot exist uncombined except after prolonged hydrolysis at high temperature in strong acid. Histological preparations, naturally enough, do not survive such treatment." (Rough translations in the footnotes supplied by RB.)] See also Brachet, 1941 (footnote 11), 88 ff. and the retrospective comments on the techniques employed in Brachet 1975 (footnote 5), 486-487.

¹⁹ See J. Brachet, 'Étude histochimique des protéines au cours du développement embryonnaire des Poissons, de Amphibiens, et des Oiseaux', *Archives de Biologie* 51 (1940), 167-202. See also Brachet 1941 (footnote 11) and 1975 (footnote 5), 488 for this dating.

²⁰ Other stains were used as well. RNase removed the color of most of the standard basic stains that reacted with RNA. For further discussion of the topic of this paragraph, see Rheinberger and Thomas, this issue.

²¹ The evidence elaborated in Brachet 1933 (footnote 4) is amplified repeatedly in papers up to and including Brachet, 'Le rôle et la localisation des acides nucléiques au cours du développement embryonnaire', *Comptes rendus de la Société de Biologie* 42 (1948), 1241-1254. New evidence along the way includes the discovery of the high RNA content of nucleoli, of small amounts of RNA interacting with the chromosomes, and evidence that the timing of the presence of RNA in these new sites was compatible with RNA serving as a precursor used in the biosynthesis of DNA. The hypothesis is definitively rejected in Brachet, *Le rôle des acides nucléiques dans la vie de la cellule et de l'embryon*, Liège and Paris: Desoer and Masson, 1952: 35-36. I am not yet sure exactly when he changed his mind, but the 1952 text suggests that it is due to his acceptance of the results of G. Schmidt, L. Hecht, and S. J. Tannhauser, 'The behavior of the nucleic acids during the development of the sea urchin egg (*Arbacia*)', *Journal of General Physiology* 31 (1948), 203-207 and C. A. Villee, M. Lowens, M. Gordon, E. Leonard, and A. Rich 'The incorporation of P³² into nucleoproteins and phosphoproteins of the developing sea

and altered old techniques, and employed new ones such as the use of radioactive tracers, he followed where they led even when it meant entering new disciplines and overthrowing fundamental beliefs about the objects of study, including his own.

The results of Brachet's exploratory experiments pointed in many directions — too many to handle all at once. After all, he was already searching for the inducer, trying to understand the synthesis of DNA, looking for the mechanisms by means of which nucleic acids influence the ontogeny of embryos, searching for the relationship between RNA and protein synthesis, investigating the mechanisms of morphogenesis, and working synthetically with a great variety of organisms on the whole gamut of issues in biochemical cytology and embryology. On top of which, the Belgian academic authorities shut down the university for almost three years in order to resist an order from the Nazis to provide a list of the Jewish personnel employed there²² and the Nazis actually held Brachet as a hostage from December 1942 to March 1943. For a period of at least five years, Brachet and his co-workers were either prevented from carrying out experiments altogether or severely limited by the need to work with jury-rigged and often rather primitive apparatus. Nonetheless, one group of new findings slowly became more and more integrated into the work. These findings involved the role of cytoplasmic RNAs in protein synthesis. I restrict attention to that line of work in the remainder of this paper because it illustrates rather nicely the exploratory character of all of his programs of research.

THE BEGINNINGS OF WORK ON PROTEIN SYNTHESIS

Brachet started using Unna stain and RNase around 1940. Rheinberger's paper in this issue spells out in detail that this was just when Claude's early results with ultracentrifuged microsomal particles were coming in. Brachet, of course, knew of Claude's work. Jeener had access to a Henriot ultracentrifuge and, under terribly difficult circumstances in war-time isolation, Brachet, Jeener, and Chantrenne combined the use of RNase and the Unna stain with ultracentrifugation. They worked on interrelated projects and, to my unpracticed eye, managed to pursue nearly every means of histochemical measurement available within their limited means.

By 1941, using amphibians, mammals, and silkworms, Jeener and Brachet found that centrifuged microsomal particles from organs that actively synthesize proteins are complexed with a variety of hormones, enzymes and proteins. Some respiratory enzymes were always fixed to the particles in the pellet. Cells taken from an organ actively synthesizing some product were usually complexed with organ-specific proteins as well.²³ This seemed to be true *in vivo* as well as *in vitro*; Chantrenne showed that when intact liver cells are centrifuged, the specific activity

urchin embryo', *Journal of Cellular and Comparative Physiology* 23 (1949), 93-112. These papers showed that Brachet's old method of estimating RNA content by means of the production of furfural, Brachet 1941 (footnote 11), 88 ff., produced an artefactual estimate of diminution of RNA in early ontogeny. The reduction in the measured quantity of furfural in sea urchins was really due to the loss of the jelly that surrounded the unfertilized eggs and which produced furfural, a result that invalidated the assay. See also Brachet 1975 (footnote 5), 487.

²² I thank R. Thomas for a clarification of the reason for which the university was closed. See his contribution to this issue.

²³R. Jeener and J. Brachet 'Sur la présence d'hormones protéiques et d'hémoglobine dans les granules a pentosenucléoprotéides' *Acta Biologica Belgica* II (1942): 447-450.

of liver proteins remained attached to the lunule of microsomal particles.²⁴ Similar results were obtained for other cells and enzymes and in additional organisms. They tentatively concluded that the co-presence of the granules and enzymes could best be explained by supposing that the synthesis of the enzyme and protein molecules depended on the presence of pentose nucleoproteins, i.e., RNA.²⁵ By 1944, they knew that merogonic haploids and various other modified sea urchin and anuran embryos (parthenogenetic, polyspermic, etc.) typically produced less RNA when compared with normal embryos. Like Caspersson and Schultz (who had similar results for drosophila) they took this as a possible indication that the underlying problem concerned nucleoprotein metabolism.²⁶

These views were supported in a variety of ways²⁷ and became more and more prominent in the papers of Brachet, Chantrenne, and Jeener during the early and mid 40s. By 1942 they had firm results showing that the nucleolus contains a considerable amount of RNA²⁸, that the ergastoplasm is especially rich in nucleoprotein granules, and that there is some RNA *in the nucleus* associated or complexed with heterochromatin.²⁹ By 1942 Jeener and Brachet³⁰ had also shown that there are distinct pentose nucleic acids in yeast cytoplasm — one in the nucleoprotein granules and one that remains in the supernatant when the granules are removed by ultracentrifugation. They report that in a growing culture, the amount of nucleic acid increases at a greater rate than cell mass or volume and that the amount of ‘free’ nucleic acid increases more rapidly than the nucleic acid in the granules; however, since the granules increase at the same

²⁴ H. Chantrenne, ‘Association de la cytochrome-oxidase à des complexes sédimentables dans le cytoplasme vivante’, *Acta Biologica Belgica* III (1943): 99-102. See also Chantrenne, ‘Recherches sur des particules cytoplasmiques de dimensions macromoléculaires riches en acide pentosenucléiques. II. Relations avec les ferments respiratoires’, *Enzymologia* 11 (1943-1944): 213-221.

²⁵ “Dès lors, leur [les ferments] présence simultanée dans les granules s’explique le mieux en supposant que la synthèse des molécules de ferments, au même titre que celle de toutes les molécules de protéines, serait liée à la présence de pentosenucléoprotéides (J. Brachet, Caspersson)” (R. Jeener and J. Brachet, ‘Association, dans un même granule, de ferments et des pentosenucléoprotéides cytoplasmiques’, *Acta Biologica Belgica* I (1941): 476-481 480-481). [From then on, their [the enzymes’] co-presence in the granules, like that of all protein molecules, is best explained by supposing that it was connected to the presence of pentose nucleoproteins.”] See also Jeener and Brachet, 1942 (footnote 23).

²⁶ Brachet 1944 (footnote 8), 476. It is perhaps worth noting that Brachet and his colleagues were keenly aware of (and consistently mentioned) the parallel work of Caspersson and his colleagues during this period which, using entirely different methods, also suggested the association of RNA with protein synthesis and localized RNA in the cytoplasm (especially ergastoplasm) and the nucleolus, and DNA in the nucleus. Brachet 1944 (footnote 8) includes an extensive if somewhat scattered account of Caspersson’s findings and hypotheses, including those associating RNA with protein synthesis. The interesting topic of the relationship between the findings (and the people) of the two groups cannot be handled in the present paper, but a particularly interesting example is worthy of note, to wit, Brachet’s discussion at pp. 73-75 of Caspersson and Schultz’s finding that the quantity of RNA in the nucleolus and the cytoplasm depend on the quantity and constitution of the DNA in the nucleus (T. Caspersson and J. Schultz ‘Ribonucleic acids in both nucleus and cytoplasm and the function of the nucleolus’, *Proceedings of the National Academy of Sciences, USA* 26 (1940): 507-515). See also Brachet 1944, 227-240 and 475-476; other discussions of the work of Caspersson and his colleagues in Brachet 1944 are easily located by using the bibliography, which lists the pages on which Brachet discusses each entry).

²⁷ J. Brachet, ‘La localisation des acides pentosenucléiques dans les tissus animaux et les œufs d’Amphibiens en voie de développement’, *Archives de Biologie* 53 (1942): 207-257, 239 and ‘Acides nucléiques et morphogénèse au cours de la parthénogénèse, la polyspermie, et l’hybridation chez les anoures’, *Annales de la Société royale Zoologique de Belgique* 75 (1944): 49-74, 69 ff.

²⁸ Brachet 1942 (footnote 27), 241-242.

²⁹ *Ibid.*, 242-246; 1944 (footnote 8), 223-224.

³⁰ R. Jeener and J. Brachet, ‘Les pentosenucléoprotéides combinés et libres au cours de la croissance des levures’, *Acta Biologica Belgica* II (1942): 273-276.

rate as total cellular nitrogen, they conclude that it is a stable and fundamental part of the cell, in contrast to the extremely labile and heterogeneous supernatant RNA, which (they claim) exemplifies disharmonic (or differential) growth in the sense of Teissier. The amount of free RNA increases more rapidly than the amount of total nitrogen (and thus, presumably, the amount of total protein) in the cell, whereas the amount of RNA bound in microsomes increases in proportion to the increase of total cellular nitrogen.³¹ Similarly, Brachet and Chantrenne³² located free nucleoprotein in amphibian eggs — behaving exactly as would be expected if it were the reserve material employed to supply the developing embryo with the constituents of DNA. That is, 60%-80% of the RNA of the virgin egg was not located in the granular particles, but was found in the supernatant after those particles were spun down — and that fraction disappeared over time, apparently in synchrony with the formation of DNA in the embryo, tracked all the way through gastrulation to the hatching of the tadpole. In spite of their difficult circumstances, they somehow managed to provide fairly full experimental support for these claims in three back-to-back papers submitted in 1943 to *Enzymologia*.³³

It is perhaps worth quoting Brachet's extended summary of his views on this issue as of late 1943 or early 1944:

Il reste un dernier point à élucider : c'est le rôle respectif de la fraction « libre » et de celle qui est combinée aux granules. Ces dernières interviennent évidemment dans la synthèse des protéines ; nous savons aussi que l'acide ribonucléique «

³¹ “Nous pouvons en conclure que l'acide nucléique des granules est un élément stable, faisant partie des constituants fondamentaux de la cellule, alors que l'acide nucléique libre, d'une extrême labilité, est responsable à lui seule de l'accroissement dysharmonique (Teissier) ou hétérogonique (Needham) de l'acide nucléique total signalé au début de cette note. ... Nous en arriverions ainsi à constater que l'accroissement de la teneur de la cellule de levure en granules à pentosenucléoprotéides est parallèle à l'accroissement de l'azote total, principalement protéique, qu'elle fixe au cours de l'évolution d'une culture. Si une relation existe, comme nous le pensons, entre l'accumulation d'acide pentosenucléique dans une cellule et la vitesse de synthèse des protéines, il est par conséquent logique d'admettre que l'acide nucléique des granules exerce seul une influence dans cette synthèse, alors que l'acide nucléique libre, fortement hétérogonique, ne peut y jouer un rôle direct” (*Ibid.*, 276). [“We can conclude from this that the nucleic acid of the granules is a stable component [lit. element], serving as one of the fundamental constituents of the cell, while the free nucleic acid, which is extremely labile, is responsible for the disharmonic (Teissier) or heterogonic (Needham) increase of the total nucleic acid mentioned at the beginning of this note. ... We thus arrive at the assertion that the increase in the number of pentosenucleoprotein granules in the cells of yeast parallels the increase of total nitrogen, mainly in proteins, fixed in the evolution of a culture. If there is a relationship, as we think there is, between the accumulation of pentosenucleic acid in the cell and the speed of protein synthesis, it is logical, in consequence, to admit that the nucleic acid of the granules alone influences this synthesis, while the free nucleic acid, strongly heterogonic, cannot play a direct role in this connection.”] The indirect references are supplied in R. Jeener and J. Brachet, ‘Recherches sur l'acide ribonucléique des levures (Microdosage, relations avec la croissance, conditions de sa synthèse),’, *Enzymologia* 11 (1943-44): 222-234., p. 226). They are to G. Teissier, ‘Croissance des insectes,’ *Travaux de la Station Biologique de Roscoff* 9 (1931): 29 ff. and J. Needham, ‘Chemical heterogony and the groundplan of animal growth,’ *Biological Reviews* 9 (1934): 79-109. See also Teissier, ‘Dysharmonies biochimiques dans la croissance larvaire de *Tenebrio molitor* L.’ *Comptes rendus de la Société de biologie* 50 (1929): 1171-1173 and *Dysharmonies et Discontinuités dans la Croissance*, Hermann: Paris, 1934.

³² J. Brachet and H. Chantrenne, ‘Nucléoprotéines libres et combinés sous forme de granules chez l'œuf d'Amphibiens,’ *Acta Biologica Belgica* II (1942): 451-454.

³³ J. Brachet and R. Jeener ‘Recherches sur des particules cytoplasmiques de dimensions macromoléculaires riches en acide pentosenucléique. I. Propriétés générales, relations avec des hydrolases, les hormones, les protéines de structure,’ *Enzymologia* 11 (1943-44): 196-212, H. Chantrenne 1943-44 (footnote 24).

libre » ne se rencontre en proportion notable que dans les cellules en voie de prolifération active : levures, embryons de poulet et de grenouille ; dans le cas de la levure tout au moins, il n'y a aucune apparence que les ribonucléoprotéides libres participent à la synthèse de protéines. On en arrive donc à penser que la fraction « libre », plus labile, interviendrait dans la synthèse de l'acide thymonucléique et la production de cophosphorylases. Cette supposition acquiert quelque crédit du fait que la teneur en ribonucléoprotéides libres décroît progressivement au cours du développement tout comme la fréquence des mitoses. L'étude d'un matériel favorable, tel que l'œuf d'oursin, permettrait de vérifier si pareille hypothèse est réellement fondée.³⁴

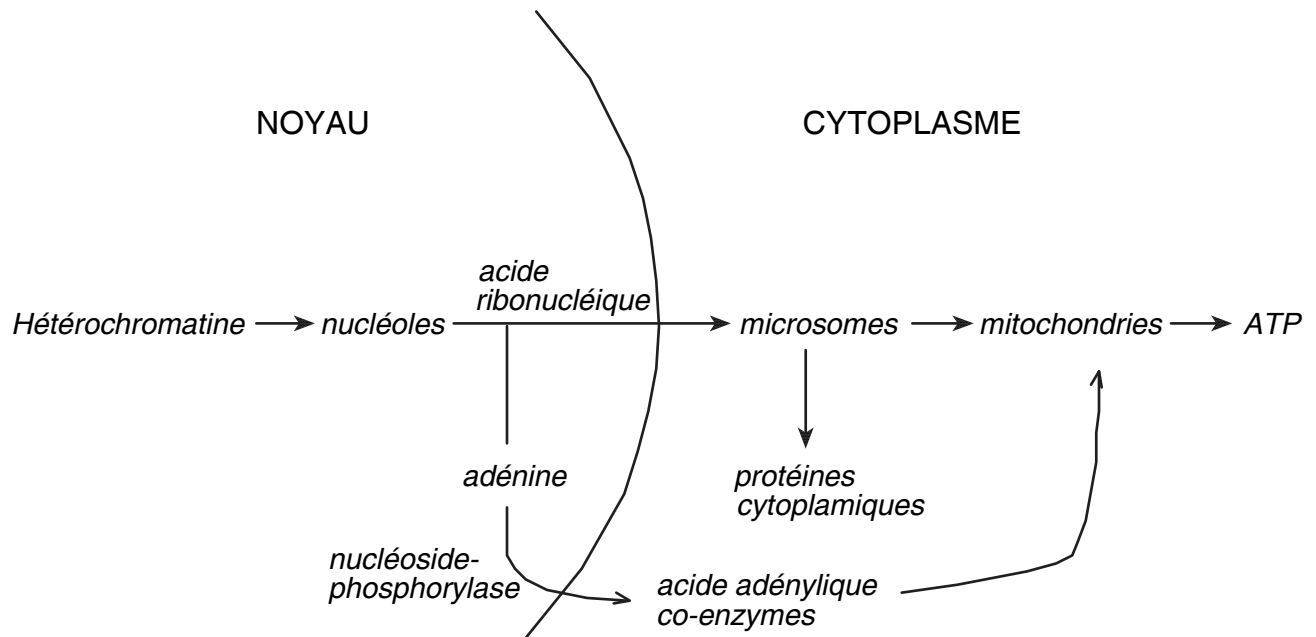
For a variety of reasons, as this quotation indicates, they believed that the RNA in nucleoprotein granules is the RNA responsible for protein synthesis. The resemblance between microsomes and TMV particles provided some support for this view, as did various theoretical considerations entering into the plasmagene hypothesis³⁵. By 1947 it was clear that the ribonucleoprotein particles had become candidates to serve as plasmagenes.³⁶ In the course of this line of work with Chantrenne, and Jeener, Brachet and his collaborators developed a biochemical schema of the processes entering into protein synthesis. As far as I know, that image, recently republished in at least four historical papers and presented here as figure one, was first published as the sole diagram of Brachet's marvelous little book of 1952, *Le rôle des acides nucléiques dans la vie de la cellule et de l'embryon*.

³⁴ “There remains one last point to elucidate, namely the respective roles of the ‘free’ fraction [of RNA] and that which is bound to the granules. These last evidently play a role in the synthesis of proteins; we also know that ‘free’ ribonucleic acid is not found in a significant proportion except in those cells that are actively proliferating — e.g., yeast, embryos of chickens and frogs. In the case of yeast at the least, it does not appear that free ribonucleoproteins play a role in protein synthesis. One thus arrives at the idea that the ‘free’ fraction, more labile, enters into the synthesis of thymonucleic acid and the production of cophosphorylases. This supposition receives some support from the fact that the quantity of free ribonucleoproteins decreases progressively during development in proportion to the frequency of mitoses. The study of a favorable material, such as sea urchin eggs, will allow one to verify whether this hypothesis is well founded” (Brachet 1944, 250) (footnote 8).

³⁵ See Jan Sapp's paper, this issue, as well as Sapp *Beyond the Gene: Cytoplasmic Heredity and the Struggle for Authority in Genetics*, New York: Oxford University Press, 1987; *Idem*. ‘Inside the Cell: Genetic Methodology and the Case of the Cytoplasm. In: J.A. Schuster and R. R. Yeo (eds.), *The Politics and Rhetoric of Scientific Method*, Dordrecht: D. Reidel, 1986, 178-202; and *Idem*, ‘Hérédité cytoplasmique et histoire de la génétique’. In: J.-L. Fischer and W. Schneider (eds.), *Histoire de la génétique*. Paris: A.R.P.E.M & Éditions sciences en situation, 1990: 231-246. See also R. Burian ‘La contribution française aux instruments de recherche dans le domaine de la génétique moléculaire’. In: J.-L. Fischer and W. Schneider (eds.), *Histoire de la génétique*. Paris: A.R.P.E.M & Éditions sciences en situation, 1990: 247-269 and 1993 (footnote 16), and S. Gilbert ‘Enzyme adaptation and the entrance of molecular biology into embryology.’ In S. Sarkar (ed.), *The Philosophy and History of Molecular Biology: New Perspectives*, Dordrecht: Kluwer, 1996: 101-123.

³⁶ See e.g., Brachet 1947 (footnote 4); ‘L’hypothèse des plasmagènes dans le développement et la différenciation’. In: *Unités biologiques douées de continuité génétique* (Paris, Éditions du Centre National de la Recherche Scientifique, 1949): 145-163; and Sapp, this volume.

Brachet 1952



This diagram is of deep interest. At the symposium presentation on which the present paper is based, both Rheinberger and Sapp argued that I had put too much emphasis on the surface parallels between the account of protein synthesis implicit in it and Crick's articulation of the 'central dogma' of molecular biology, to wit, that DNA and RNA are carriers of the information that specifies the construction of proteins and that information does not go back from proteins to the nucleic acids³⁷ There is considerable justice in their warning. Brachet's diagram is clearly biochemical in inspiration and his argument is couched in terms of the enzymatically fostered formation of compounds and the energetics of the relevant reactions (thus requiring employment of biochemical energetics in the analysis). In all of this there is no hint of a notion of information or of colinearity between the nucleotides of DNA or RNA and the amino acids on the protein produced under genetic control. In spite of this, the comparison of Brachet's scheme and that of Crick some six years later³⁸ lends some support to the claim that I was trying to articulate, which is that Brachet's interpretation illustrates a significant *convergence* between analyses built on quite divergent styles of investigation and quite divergent perspectives. Brachet's perspective was rooted in biochemistry and (bio)chemical embryology;³⁹ he addressed their problems by use of the problems of these disciplines by use of their tools, supplemented where possible by such new ones as radioactive tracers. Thus his investigative pathway was

³⁷ F. H. C. Crick 'On protein synthesis', *The Biological Replication of Macromolecules. Symposia of the Society for Experimental Biology* **12** (1958): 138-163 and 'The central dogma of molecular biology', *Nature* **227** (1970): 561-563.

³⁸ Crick 1958 (footnote 37).

³⁹ See Fantini 1978 (footnote 6).

quite different than those of Watson and Crick, Jacob and Monod, Delbrück and the phage workers, and others on the usual lists of the pioneers of molecular biology. These pioneers typically employed tools imported from physics in combination with the new methods of genetic analysis applied to microorganism; their findings were based largely on the use of microorganisms (primarily procaryotes, 'lower' eucaryotes, and phage).

Against this backdrop, it is perhaps not entirely surprising that the Rouge Cloître group, with its more exploratory ethos, its embryological and biochemical traditions, and its use of extremely diverse organisms (many of which were not well suited to genetic analysis), is not included in the standard histories of molecular biology, all of which emphasize the effects of the invasion of physicists into biology after WW II and concentrate on the more familiar pathways leading to molecular *genetics*. If it is right to characterize the findings established in these sharply divergent investigative pathways as achieving something like convergence, we have here an important instance of what (following Bill Wimsatt and many others) I have occasionally called *triangulation*.⁴⁰

Brachet and his colleagues developed strong evidence of their own for the claim that nuclear genes control the synthesis of enzymes and proteins, greatly reinforced in the next decade by their work on *Acetabularia*.⁴¹ Although they connected their projects with some of the work on the genetics of microorganisms that helped to shape the development of molecular genetics,⁴² they were more concerned with biochemical analyses and characterizations of such substances as 'free RNA', substances that were later recognized as essential components of 'informational' molecular genetics and of crucial importance for *in vitro* biochemical analyses of protein synthesis. Ultimately, these three avenues of investigation, originally independent, ended up being cross-collated so that one could be sure that their distinctive protocols and (theoretical) descriptions actually picked out and referred to the same molecules. Getting to this point was not easy; this is obvious as soon as one pays attention to the confusion about the various 'species' of RNA — sRNA (Zamecnik *et al.*), free RNA (Brachet *et al.*) mRNA, rRNA, and tRNA).⁴³ It

⁴⁰ See, e.g., Burian 1993 (footnote 16), 399, 403.

⁴¹ See Burian, 'Underappreciated Pathways Toward Molecular Genetics as Illustrated by Jean Brachet's Chemical Embryology'. In: S. Sarkar (ed.), *The Philosophy and History of Molecular Biology: New Perspectives*, Dordrecht: Kluwer, 1996: 67-85.

⁴² See, for example, the last section of Brachet 1944 (footnote 8), entitled, "Gènes, Morphogénèse et Biochimie", 472-478), or the following later quotation: "Il ne semble pas douteux non plus que les gènes présent dans l'euchromatine contrôlent la synthèse d'enzymes spécifiques, dont chacun est requis pour accomplir une étape dans une réaction complexe, conduisant à la synthèse d'un composé organique : l'analyse génétique et biochimique de la synthèse du tryptophane, de l'acide nicotinique, de la méthionine, etc., chez *Neurospora* (BEADLE et ses collaborateurs [G. Beadle, 'Genes and biological enigmas'. In: G. A. Baitsell (ed.), *Science in Progress*, New Haven: Yale University Press, 1942: 184-249.] en fournit une preuve convaincante" (Brachet 1952, p. 112) (footnote 21). ["There is no longer any doubt but that the genes present in the euchromatin control the synthesis of specific enzymes, each of which is required for the completion of a step in the complex of reactions yielding the synthesis of an organic compound: genetic analysis and biochemical synthesis of tryptophane, nicotinic acid, methionine, etc. in *Neurospora* (BEADLE and his collaborators [Beadle 1949]) have provided a convincing proof of this."]

⁴³ Extreme care is needed in parsing such claims as this. For example, Zamecnik's 'soluble RNA (sRNA) turned out to be the same as Crick's 'adaptor', which we now know as tRNA (Rheinberger 1993 (footnote 7) and in press (footnote 7)). This is clearly distinct from Brachet's 'free RNA', which represented some 60% or more of the RNA in virgin amphibian eggs; this, of course, is mRNA. But as Thieffry shows in this issue, there were significant disagreements between the Rouge Cloître and the Pasteur groups over the identification of mRNAs because of the latter's early insistence that the half life of mRNA is extremely short. Even in the best of cases, the definitive identification of an 'epistemic

took great effort to disentangle exactly when the different analyses picked out the same molecules.

The very fact that work in three such different styles (and with aims as diverse) as Brachet's, Crick's, and Zamecnik's⁴⁴ could be brought into concordance in about one decade serves as an important marker of the stabilization of the entities and phenomena in the enormous domain covered by these three distinctive research programs. One of the most important tasks in the philosophy of biology — indeed, the philosophy of science generally — is to understand how we achieve concordance in the interpretations of the findings of workers who, in Rheinberger's terminology, work with such different 'epistemic objects' as those that preoccupied these three key figures. It is in handling such topics as this that the philosophy of experiment will find its liberation from the excessive theory-centrism, now waning, of recent philosophy of science.

SOME CONCLUDING THOUGHTS ON EXPLORATORY EXPERIMENTS

I hope that this study has helped illuminate the style of Jean Brachet's early work, including that done in collaboration with Raymond Jeener and Hubert Chantrenne. I also hope it will help to stimulate wider interest among philosophers of science in what I have called

object' (a stably reproducible entity or outcome of an experimental procedure known to its partisans by some particular identifying description) with another (identified via significantly different procedures and descriptions) is a major accomplishment that depends on the outcome of long series of experimental investigations and debates among the relevant scientists. Rheinberger emphasizes the differences that go into the separate identifications, while I emphasize the possibility that seemingly independent epistemic objects, if they survive sceptical scrutiny, will turn out to be the same thing identified in different ways. Each of us is probably guilty of a certain degree of overemphasis. For our overlapping views on these matters see Rheinberger 1993 (footnote 7); 1995 (footnote 16); and 'Experimental Complexity in Biology: Epistemological and Historical Remarks' *Philosophy of Science* 64 (Proceedings) (1997); and Burian 1993 (footnote 40); 'Comments on Hans-Jörg Rheinberger's 'From Experimental Systems to Cultures of Experimentation'. In: G. Wolters and J. Lennox, in collaboration with P. McLaughlin (eds.), *Concepts, Theories, and Rationality in the Biological Sciences: The Second Pittsburgh - Konstanz Colloquium in the Philosophy of Science*, Konstanz and Pittsburgh: UKV-Universitätsverlag Konstanz and University of Pittsburgh Press 1995, 123-136; and 'Comments on Complexity and Experimentation in Biology', *Philosophy of Science* 64 (Proceedings) (1997).

⁴⁴ The issues concerning style — and in characterizing the different styles of these three investigators — are far too large to pursue on this occasion. Nonetheless, a thumbnail characterization will be useful here. Brachet sought to obtain a step-by-step account of protein synthesis and, more generally, of the development of embryos by localizing and following biologically significant molecules at various points in unicells, in embryos, and in the cell cycle. By characterizing the interactions and effects of those molecules he pursued a kind of biochemical *Entwicklungsmechanik* built in considerable part on exploratory experimentation. Crick, notoriously a theoretician, sought to minimize dependence on messy experiments by first doing as much as possible to limit the information required from experiments. Ideally, he sought to accomplish this by seeking a key experiment that would discriminate sharply between two previously developed hypotheses, with other alternatives excluded on theoretical grounds. And Zamecnik sought to build an *in vitro* system adequate to produce a biochemical analysis that would dissect each step of protein synthesis. For him, the *in vitro* work would then have to be cross-checked *in vivo*, while for Brachet *in vivo* work was the primary issue and *in vitro* work was a necessary crutch to be distrusted and used with caution. As Sapp, this issue, points out, Brachet, like Ephrussi, felt that embryology had to be done, in the end, in embryos. This attitude carried over even into his work in molecular genetics, biochemistry, and embryology. The best guide to Zamecnik's work, with its contrasting reliance on *in vitro* experiments is Rheinberger (in press), which builds on a number of his earlier papers.

exploratory experiments and the roles they play in the development of scientific knowledge and scientific theories. It is to the latter topic that I turn in the closing section of this paper.

A great deal of the research discussed above involved *exploratory experiments*. New techniques, wedded to older ones, were typically put to use to uncover the unknown places in which, and the unknown sequence in which nucleic acids and various, enzymes, hormones, proteins, and other substances are present in particular cells or cellular compartments, to follow those molecules through the stages of the cell cycle and the ontogeny of a wide variety of organisms, and to learn the effects of perturbing the cell or organism so as to alter the availability or composition of the nucleic acids. Among the important perturbations, fractionation or separation of different cellular components, e.g., by ultracentrifugation, was of particular importance. Blocking of the synthesis of RNA or DNA by specific inhibitors also became feasible after the war. The primary purpose of such experiments was to find correlations between the presence of nucleic acids at particular times and places and the ensuing biochemical, physiological, and morphological changes, with the ultimate aim of understanding the contributions of nucleic acids and related substances to differentiation, growth, morphological change, and the entire ontogenetic process. Many techniques were employed to cross check the locations, concentrations, and identities of various substances, most especially the nucleic acids, and to determine the differences that arose when they were present in different concentrations or at different times, when a step into which they entered was blocked, or when they were altered in some way. By this means, Brachet and his colleagues sought improved understanding of phenomena and sequences of change already partially understood. In the process, some entities (such as the free RNA in the supernatant) seemed to serve quite different functions at various stages of the research, so much so that they seemed to acquire new identities. In Rheinberger's terminology, as the researchers' background knowledge and the apparent functions of various substances changed, those substances became different 'epistemic objects'. Nonetheless, the stabilization of the protocols for locating particular molecular species and for identifying, and reidentifying the molecules thus localized made it possible to check whether or not, e.g., 'soluble RNA' (Zamecnik) and 'free RNA' (Brachet) were the same thing and, eventually (after the former turned out to be Crick's adaptor and was transformed into tRNA), whether the latter could be the messenger RNA proposed by Jacob and Monod.⁴⁵

One of the key ways of establishing that the entities (in the present case, molecules) picked out by use of different protocols are the same is by establishing stable ways of locating them in space and time and applying appropriate tests to them only after such localization has been accomplished. This was precisely the function of combining the use of the ultracentrifuge with the cross-checked histochemical and enzymatic techniques nicely exemplified by the work with the Unna stain. The exploratory experiments performed by Brachet and his Rouge Cloître colleagues served to localize and characterize the nucleic acids in such a way that, as it turned out, the results proved to be relevant to the experimental and theoretical analyses of such other investigative traditions as those of Zamecnik, Watson and Crick, Monod and Jacob, and the phage group.

This sort of transfer of knowledge was facilitated by the fact that spatio-temporal localization need not, as such, require a strong commitment to particular functional

⁴⁵ F. Jacob and J. Monod, 'Genetic regulatory mechanisms in the synthesis of proteins', *Journal of Molecular Biology* 3 (1960): 318-356 and 'On the regulation of gene activity: b-galactosidase formation in *E. coli*', *Cold Spring Harbor Symposia in Quantitative Biology* 26 (1961): 193-211.

characterizations of the entity (or entities) localized. In the cases discussed in this paper, even though the tasks at hand required extreme refinement in the development and cross-checking of techniques to avoid artefacts, the recognition and localization of the distinct classes of nucleic acid was achieved without depending on particular hypotheses about the functions of those distinct substances. In virtue of this relative independence of the identification of the molecules from theoretical and functional characterizations of their roles in the life of the cell or in ontogeny, Brachet and his colleagues provided appropriate means for testing a great variety of hypotheses about their detailed molecular structures, behaviors and functions. Accordingly, although there were sometimes strong shifts in the characterizations of particular classes of molecules and of their behavior, the key to a number of significant advances was the Rouge Cloître group's great emphasis on the use of a great variety of histochemical and other means of localizing the molecules and on sufficient cross-checking by multiple techniques to be reasonably confident that artefacts were eliminated. Thus this exploratory work made it possible to attack the issues regarding the functions and causal roles of molecules and substances that could be produced more-or-less at will and then examined by multiple techniques from a great variety of theoretical perspectives. This emphasis on localization and pursuit (which carried over to the use of new techniques such as pulse-chase experiments) characterized much of the work that I have examined in this paper.

This approach, set in a careful spatio-temporal analysis of the correlated biochemical, morphological and ontogenetic changes in the cells and organisms studied, was joined to an exceptionally broad knowledge base built by Brachet and his colleagues about the biochemistry, ontogeny, and morphology of various organisms. This extensive background knowledge was employed not only to address such specific issues as the dispute between Godlewski and Loeb with which Brachet began his career, but also to attack broader problems such as ferreting out the modes of synthesis of the nucleic acids, the roles of nucleic acids in ontogeny, and the means by which cells synthesize proteins.

The general point can be put more abstractly in a way that is useful in the philosophy of science. A key feature, crucial to the success of the immensely elaborate series of interconnected experiments discussed in this paper, was the firmly rooted technical base for reidentifying and localizing entities such as the nucleic acids in multiple ways. Cross-checking of the biochemical constitution and spatio-temporal localization of the nucleic acids was fostered by the enormous diversity of techniques for characterizing and following them. These included their responses to vital stains, their entry into certain characteristic biochemical reactions, the way they were separated by ultracentrifugation, the conditions in which they were synthesized or degraded, and various morphological criteria based on the complexing of DNA and RNA with other substances to form stably configured entities such as chromosomes and ribosomes. It would be difficult to overestimate the importance of such a broad technical base (expanded even further in the present case by the use of a great variety of experimental organisms) for sound exploratory experimentation. At the heart of the matter is the need for a battery of technically adequate means for cross-checking different techniques, one against another, for reidentifying a 'thing' or process. For some five decades, the Rouge Cloître group was able to follow thymonucleic acid and zymonucleic acid from their early identities as animal and plant nucleic acids, relatively uninteresting molecules playing some sort of buffering role, into the new world of molecular biology, where they became the fascinating and ubiquitous DNA and RNA molecules, carrying genetic messages, forming various parts of the machinery for transporting amino acids, for assembling polypeptide chains, and translating genetic messages into protein. In spite of the

radical ways in which the accounts of the nature, composition, location, and synthesis of DNA and RNA changed during that long period, by localizing different classes of these molecules and following (and reidentifying) them wherever they led, the Rouge Cloître group was able to use those substances as an Ariadne's thread to guide them in developing the new understandings of the vastly expanded territory that was opened to scientific exploration. The fruitfulness of their work depended, I believe, on the fact that their experimental program was built on the pursuit of the roles of particular substances in the development of the organism considered as a larger whole.

In our roles as historians and philosophers of science, it is important for us to improve our understanding how exploratory experimentation, such as that discussed in this paper, works, with its cross-checking of spatio-temporal localization and physical identity. As I have shown, such experimentation can be crucial in providing the means of stabilizing transtheoretical claims about the characteristics and behavior of 'theoretical' entities such as (what we now call) mRNA and tRNA. If this approach is sound, it should yield a solution to one of my puzzles about Rheinberger's studies of exploratory experimentation, namely, how to establish contact between researchers pursuing distinct epistemic objects which have been produced by different experimental groups working within different disciplines and theoretical traditions. The key to the solution of this puzzle is to establish the means for localizing entities (including 'epistemic objects') in space and time without depending on the theoretical or functional identities assigned to them, however provisionally. In this way, we gain access to those entities in ways that do not depend wholly on the specific disciplinary or theoretical background of the experimenters who initiated the work on those objects. In this way we will be able to compare different, seemingly incompatible, epistemic objects via the kinds of cross-checking illustrated by the work of the Rouge Cloître group. In this way, we can fully appreciate the most important difference between mitogenetic rays and the various nucleic acids, namely, the independent localizability of the latter. For those of us working in the history and philosophy of biology, the clarity with which Jean Brachet and his colleagues pursued a project of this sort adds a valuable side benefit to the study of this intrinsically fascinating work.

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