Claude Bernard and Pancreatic Function revisited after 150 years

A.C. Rodriguez de Romo & J. Borgstein

Summary

In 1848, Claude Bernard described the lipolytic function of the pancreas. His experimental procedure was original and as he always affirmed, easily replicable. In this study we repeated the original Bernardian experiments for emutsification and saponification of fats, following the original descriptions from his labororatory manuscripts. The results were astonishingly similar to those obtained by Bernard, confirming the validity of his experiments and conclusions, as well as emphasising again his original concepts on the importance of experimental repeatability in scientific medicine. Considering the surgical difficulties we encountered, we must conclude that Claude Bernard required great manual dexterity to obtain these results without the benefit of modern technology and anaesthesia.

Resume

En 1848, Claude Bernard decouvrit que le pancreas emulsionne et saponifie les graisses. La methode experimentale etait tres originate et Bernard a toujours dit qu'elle etait facilement reproductible. Ce travail reproduit des experiences bernardiennes de /'emulsion et de la saponification de la graisse. L'idee est originate car nous avons pris les experiences de son cahier de laboratoire. Nous avons eu les mimes resultats que Claude Bernard. La validite de ces experiences, ses conclusions etses idees surla reproductibilite experimentale, on ete confirmees. D'apres les problemes chirurgicaux que nous avons eu, nous pensons que Claude Bernard etait tres doue dans une epoque qui n'avait pas l'anesthesie et la technologie de nos jours.

Introduction

Claude Bernard (1813-1878), the 19th century "father" of modern physiology is well known for his studies on the liver, his speculations on homeostasis or *"milieu interieur"* and especially for his lucid philosophical analysis of the experimental method (1), which remains a classic, and should form essential reading for any physician embarking on research.

It is not so well known now that he also made important discoveries on the function of the pancreas, and carried out extensive experiments to confirm this. He carried out extensive research on the pancreas to discover its function which was previously unknown, though much speculated on in his time.

In 1848 Claude Bernard at the age of 35, made his first major scientific discovery; that the pancreas produces a substance capable of

Ana Cecilia Rodriguez de Romo, Department of History and Philosophy of Medicine, Faculty of Medicine, National Autonomous University of Mexico (UNAM)
Johannes Borgstein, Department of Surgery, Faculty of Medicine National Autonomous University of Mexico (UNAM)
Correspondence : Dr A.C. Rodriguez de Romo, Edificio 11, Depto. 903, Villa Olimpica, Tlalpan 22 Mexico DF 14020, E- Mail: borg@ survidor. unam. mx

emulsifying and saponifying neutral fats ferment *(ferment emulsif et saponifiant)* and which we now know as pancreatic lipase enzyme. The discovery not only explains part of the exocrine function of the pancreas, but also the mechanisms of digestion and absorbtion of dietary fats, two processes which were entirely unknown at the time. These experiments are among the most elegant of his studies on digestion, and Bernard himself refers to them as the starting point of his epistemological and methodological reflections. It is important to point out however that the description of the discovery in his later writings and reflections differs considerably from that encountered in his laboratory notes.

To study in greater detail the epistemological conditions of this discovery, we analyzed and repeated the experiments found in the research protocol and laboratory notes written by Bernard almost exactly 150 years ago, following as closely as possible the methods originally described, in this crucial experiment on the pancreatic exocrine function.

The manuscripts of Claude Bernard

Bernard's copious laboratory notes give us a unique insight into the workings of the mind of one of the most prominent researchers of all time, as well as providing a window on the conditions of 19th century science and research.

We may examine how, with little else but his curiosity, a scientist focuses his attention on a specific problem, and arrives at a solution.

The main objective of this paper; the reproduction and analysis of this first important experiment was only possible because of Bernard's habit of keeping laboratory notebooks in which he described his experiments in minute detail, which allows them to be accurately repeated.

The original notebooks which cover a period from 1844 to 1878, the year of his death, are kept in the archives of the College de France, and their study may vary from the very easy to the extremely complex, due to Bernard's occasional habit of using various notebooks at the same time, while his handwriting varies from very clear to almost illegible. They are filled not only with details of experiments but also with hypotheses, ideas and philosophical reflections. The physiologist guarded them carefully and kept them with him. After his death they were collected from the house in the Rue des Ecoles by his former student Arsene d'Arsonval (1851 -1940), who preserved them at his country house where they were discovered in 1949 by Robert Courrier (2), then secretary of the Academy of Sciences. Finally in 1967, the medical historian Mirko Grmek published a catalogue of the Benard manuscripts (3), and made this valuable material accessible for study.

"Tallow from the Candle"

Claude Bernard started his medical studies in 1834 and completed them in1840, after which he left clinical medicine to dedicate himself to full time research. He did not produce any important work until 1846, when he took over an interest in the digestive processes from his mentor Francois Magendie (1783-1855).

Bernard collected various digestive fluids which he put in contact with different types of carbohydrates and proteins, to observe the digestiveaction. In February 1848, hefirstobtained pancreatic secretion after successfully producing a pancreatic fistula in a dog; an operation whose success had hitherto eluded him in a series of 22 animals which all died from peritoneal complications. He records that as soon as he had obtained sufficient pancreatic fluid, he embarked on a series of tests of its effects on sugars and what he called "nitrogenated substances" (4) (proteins). Then, without apparent reason, he combined a little tallow from the candle on his

work table with the pancreaticjuice and observed its emulsification! (5) Luck was on his side, for though candle tallow did not normally constitute part of the diet, it did contain triglycerides (it should be remembered that at the time most candles were made from boiled animal fats) susceptible to emulsification by pancreatic lipase (6). The discovery seems to have been completely intuitive and fortuitous. Bernard had not considered a study on the digestion of fats, for his protocols mention only dietary carbohydrates and proteins, while he did not even have any dietary fats amongst his reagents (there were only solutions of albumin and starch to test the glucolytic and proteolytic activity of the pancreatic secretions).

The detail of the candle tallow does not appear in any of Claude Bernard's published articles, and only emerged in a careful analysis of his experimental notebooks (7).

He then tried pancreatic secretions and macerated pancreas on different types of fats to confirm his discovery of the lipolytic activity of the pancreas. He carried out multiple experiments and indicated their application in human pathophysiology, while later using the example to illustrate his ideas on the scientific counter-proof (8).

The discovery was published in 1848 (9), with nine versions of the same article appearing in other journals (10), Bernard, then 35 years old had until that time not produced anything of interest. This paper earned him the prize for Experimental Physiology from the Academie des Sciences for the year 1848 and the red ribbon of the Legion d'Honneur. It also signified the start of a brillant scientific career.

The experiments 150 years later

Claude Bernard maintained that the validity of his conclusions was partly based on the reproducibility of his experiments. In identifying the cause or determinism of phenomena he gave more value to reproducibility than to statistics; it must be possible to reproduce the same phenomona underthe same experimental conditions. He always maintained that his studies on the lipolytic activity of the pancreas were simple to repeat. It should therefore be possible to obtain similar results to Claude Bernard, under the same experimental conditions.

We repeated with a few minor modifications, the experiment Bernard carried out on Saturday 25th March 1848 (11), and those of April the same year (12), using various other fats.

Bernard describes in detail his difficulties in producing a pancreatic fistula, and his first 22 dogs died from abdominal complications, until he managed to introduce a suitable silver canula into the pancreatic duct and obtained sufficient secretion for his subsequent experiments.

The experiment carried out by Bernard after finally obtaining pancreatic juice, was described in his notebooks as follows:

"Du sue pancreatique pure, 1/2 gramme environ auquel on a ajoute environ 5 centigrammes de suifde chandelle. Apres 8h. de digestion continue, le liquide est tres nettement alcalin, il s'est forme une emulsion blanchatre parfaitement homogene, le liquide ne surnage pas du tout au froid, au chaud non plus, seulement /'emulsion est fine comme du lait et ne presente pas du tout de grains. IIy a done une action singuHere du sue pancreatique sur la graisse. Le liquide emulsionne etait reste tres alcalin. II faudra fake a ce sujet d'autres experiences comparatives. De l'eau d'amidon ajoute le surlendemain n'a pas ete transformee apres 48h. Cela vient-il de ce que la matiere organique est unie a la graisse? Le liquide n'a pas contracts de mauvaise odeur" (13)

He distinguishes furthermore in his experiments, between «natural» pancreaticjuice

and «artificial» pancreatic juice (macerated pancreas).

It is important to point out that Bernard was not precise in the quantities used, and though he occasionally mentions cubic centimetres, usually he simply mixes his reagents. We were unable to obtain candles made of animal fats, as they are mostly made from paraffin wax now, and therefore used only edible fats for the experiment. We obtained pancreatic secretions and pancreatic tissue from two dogs programmed to be sacrified for an independent, unrelated study on osteosynthesis.

Methods

Two mongrel dogs weighing approximately 7 kilograms, were anaesthetized using intravenous pentobarbital induction and subsequent inhalation anaesthia. They were then operated on using a standard longitudinal abdominal incision. The duodenum was identified and incised longitudinally to locate the pancreatic duct, which was canalised with a small intravenous canula, and the pancreatic secretions were collected in a 5ml syringe.

Subsequently the complete pancreas was removed, and sections macerated with saline solution, in a mortar. The pancreatic juice and the macerated pancreas were combined with different edible fats and observed during 24 hours.

Results

In the first animal approximately 5ml of pancreatic secretion was obtained, but it was mixed with bile and had to be discarded, while from the second animal we obtained with difficulty, 2ml of pancreatic secretion. (Bernard does not mention any stimulation of pancreatic secretion). The pancreatic duct measures less than 1 mm in external diameter and it must have required considerable surgical dexterity on the part of Bernard to canalize the duct with a fine silver canula.

Test tube with "natural" pancreatic secretion.

To reproduce the experiment 9 described on Saturday 24 March 1848, we combined a little lard (tallow from animal fat candles being practically unobtainable now) with the pancreatic secretions. The tube was observed until the next day when we encountered a thick homogenous odourless emulsion.

Test tubes with "artificial" pancreatic secretion (macerated pancreas)

In the way described by Bernard, we prepared three test tubes with approximately 2ml liquid of macerated pancreas ("artificial pancreatic secretion"), towhich were added a small quantity (0,5g) of either lard, butter or vegetable oil (14). Three other tubes were prepared with saline solution and the same quantity of edible fats. (Claude Bernard did not use such a control group, mixing the fats instead with other animal liquids such as serum, semen, cephalospinal fluid and bile, none of which according to him produced any emulsif ication). All the tubes were shaken and kept at 35°C in a water bath for 30 minutes. The pH at this time was measured as 8 in each tube. Ten minutes later all three tubes were already slightly emulsified while the control tubes showed two separate unmixed phases of liquid and fat. The tubes were carried in the pocket of a labcoat (Bernard carried them in the pocket of his coat) and four hours later contained an homogenous emulsion. The most evident being the butter, followed by the lard and then the oil. The tubes were kept warm for 24 hours.

The next day, 24 hours after the start of the experiment, the tubes were again immersed in a water bath at 35°C. In the tube with butter, the emulsion was exactly as described by Bernard; as milk, while the tubes with lard and oil were

sligtly less emulsified than the butter, though the difference was very subtle.

On opening, the tube with butter dissipated a strong rancid smell of butyric acid, and the pH was found to be 5. Bernard records textually :

"le melange etait tres acide au papier tournesol et exhalait fortement l'acide butyrique" (15);

The rancid smell was present to a lesser degree in the othertubes, though more noticeable in the lard than in the oil.

Analysis of Results

All the results obtained were as described by Bernard. We observed the same emulsion, the same pH, the same odour, and thus the same saponification. The argument for reproductibility under similar experimental conditions is valid.

When Bernard published his findings, and was questioned by some of his contemporaries, he suggested they repeat the experiment, which some of them considered impossible, especially the surgical part. They may have been partly right, for it required considerable surgical skill and perseverance to obtain sufficient pancreatic secretion (16).

Furthermore, we knew what would happen, but Bernard had initially no idea. He would have considered us, in his words, "false experimenters" (17), which is to say, once we have designed the theory we design the experiment to confirm this theory.

Bernard often worked in the opposite direction, experimenting to see what would happen. Probably he continuously analyzed, consciously or subconsciously, a number of possible hypotheses as he worked, trusting partly to chance to indicate a direction, and hitting in this case upon the tallow of a candle on his work table to test his pancreatic fluid on.

He did not however divulge the real method of his discovery, and never included the crucial candle tallow episode in his publications. Grmerk says that the descriptions of scientific discoveries are always incomplete and therefore partially false.

The imperfection of historical descriptions are (fortunately) more often procedural than factual, and may be mainly due to the internal processes of scientific discovery, and the rigid systems of scientific communication. The researcher must find an acceptable method to make his discovery understandable for himself and others: a method wich does not always follow the strict chronological order of the discovery. Though this does not imply a deliberate attempt to mislead. The historian (re)creates the history on the basis of a conclusion drawn from researches, but there are many unwritten sources which play an important part in the historical development; feeling, intuitions, incidental findings, prejudices, luck (18). Claude Bernard probably considered the details of the candle tallow insignificant, or perhaps even a distraction from the importance of the discovery.

What motivated Claude Bernard to carry out this particular experiment, and how he carried out his experiments in general, is a study in itself. He started his investigations on digestion in 1843. By 1848 he had collected a number of observations related to fat digestion which are described elsewhere (19): unrelated phenomena without apparent explanation, and often widely separated in time: information which remained dormant in his mind until the moment of this experiment, when isolated observations were suddenly linked in his subconscious, and motivated the apparently senseless application of pancreatic fluid to candle tallow. Once this fortuitous observation had been confirmed, he tried various fats with pancreatic fluid and established the elegant proof which may be repeated by any interested experimenter to arrive at the same conclusions.

Conclusions

The Bernardian experiment with candle tallow seems in many aspects more of a game than a scientific experiment. Umberto Eco's principal character Brother William (20) and Dupin the investigator in the Morgue Street murders (21) have much in common with the investigations of Claude Bernard. The first has to clear up the mystery of the library, the second a strange murder, the third the mysteries of digestion. All three try to construct a logical structure of the various apparently unrelated elements which make up the phenomena under examination. At a certain point, a flash of intuition suddenly reveals the solution to a problem which had defied logical interpretation.

In the light of modern research, there remain a number of unanswered questions.

How was all this research carried out:

1. without apparent funding, except the money from his wife's dowry, which she seemed to have guarded jealously (22)

2. without previous ideas of the function of the pancreas. He was essentially reaching in the dark

3. without anaesthesia. The use of ether and chloroform for anaesthesia was described between 1847 and 1849 (23) (although Crawford Long had used ether as early as 1842, his results remained unpublished), while nitrous oxide was first applied in 1846 by William Morton and John Collins Warren. So the delicate surgery of producing a pancreatic fistula with a pancreatic duct measuring less than 1 mm in diameter was carried out without anaesthesia and with only very primitive instruments and apparatus at his disposal (24). Admittedly it took him 22 dogs before he got it right, and it is small wonder that Mme Bernard was a generous contributor to the Animal Society, an early forerunner the animal protection societies (25).

These are some of the questions the modern research worker, troubled by lack of funds, or waiting for some piece of highly specialised equipment to be delivered, would do well to consider more closely.

In research, the idea is basic, *Everything, first and last, leads back to the idea; the idea is the essence of all reasoning and all invention* (26), and where there is a good idea and a creative approach to research, funding is never a major obstacle.

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Biographies

Ana Cecilia Rodriguez de Romo, medical degree from the National Autonomous University of Mexico (UNAM). Masters and Doctorate in History and Philosophy of Science (Nouveau Doctorat) from the Sorbonne, University of Paris, Post Doctorate in History of Medicine in Johns Hopkins University. At present, Professor of History of Medicine at the National Autonomous University of Mexico and President of the Mexican Society for the History and Philosophy of Medicine. Published 40 scientific articles and 2 books. Principal areas of interest are the history of medicine in Mexico during the 19th and 20th centuries.

Johannes Borgstein, medical degree from the University of Groningen, The Netherlands. Postgraduate studies in Oxford, London and Mexico City. At present, Professor of Surgery at the National Autonomous University of Mexico (UNAM), researcher at the National Respiratory Diseases Institute in Mexico City. Published 48 scientific articles, 2 books on surgery, 3 books in press.