

## THEMATIC REVIEW

# 100 years of the Warburg effect: a historical perspective

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This paper forms part of a collection celebrating 100 Years of the Warburg Effect and Cancer. The guest editors for this collection were Lois Mulligan and Pascale Bossard.

## Abstract

Otto Warburg published the first paper describing what became known as the Warburg effect in 1923. All that was known about glucose metabolism at that time was that it occurred in two stages: (i) fermentation (glycolysis) in which glucose was converted to lactate, which did not require oxygen, and (ii) oxidative metabolism, in which the carbon atoms derived from glycolysis were fully oxidized to carbon dioxide, which did require oxygen. Warburg discovered that most tumour tissues produced a large amount of lactate that was reduced but not eliminated in the presence of oxygen, while most normal tissues produced a much smaller amount of lactate that was eliminated by the provision of oxygen. These findings were clearly well ahead of their time because it was another 80 years before they were to have any major impact, and even today the mechanisms underlying the Warburg effect are not completely understood.

### Key Words

- ▶ cancer
- ▶ glucose uptake
- ▶ glycolysis
- ▶ history
- ▶ Otto Warburg
- ▶ Warburg effect

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## Introduction

I was delighted and honoured to be asked to write this introductory chapter for a collection of papers dedicated to the 100th anniversary of the original description of the Warburg effect. I will start by setting the background to Warburg's discoveries originally published in 1923–1924 (Warburg 1923, Warburg & Minami 1923, Warburg *et al.* 1923, 1924), before describing what he found. Finally, I will attempt to assess the impact that his findings have had on modern research into tumour cell metabolism.

*A note on sources:* the original publications by Warburg from 1923 to 1924 are in German and unfortunately were not readily accessible to the author, so I relied instead on the English summary of a lecture given by Warburg at the Rockefeller Institute in the autumn of 1924 (Warburg 1925b). This states in a footnote: 'the address affords so admirable a résumé of the work of Professor Warburg that it may be of interest to those who do not have access to

the German literature'. For biographical details of Otto Warburg, I relied mainly on the extended obituary written by his very appreciative PhD student, Sir Hans Krebs, which is available on the Royal Society website (<https://royalsocietypublishing.org/doi/10.1098/rsbm.1972.0023>).

## Otto Warburg – early life

Otto Heinrich Warburg was born in Freiburg im Bresgau, Germany, in 1883. He was the son of Emil Warburg, then Professor of Physics at Freiburg University. His father's parents had been orthodox Jews and, despite the fact that his father converted to become a Protestant as an adult, this did cause Otto some problems much later during the Nazi era. In 1895, his father became Professor of Physics at the University of Berlin, clearly one of the top jobs in physics

within Germany. The young Otto was therefore exposed during social events at home to many famous German scientists, including Walther Nernst, Jacobus van't Hoff, Max Planck, Albert Einstein and Emil Fischer. Fischer was the 1902 recipient of the Nobel Prize in Chemistry, who, amongst other achievements, proposed the 'lock and key' hypothesis of enzyme--substrate interaction to explain how enzymes could speed up the rates of reaction in a stereospecific manner. Otto began his undergraduate studies in chemistry in Freiburg in 1901, finishing with a doctorate under the supervision of Fischer in Berlin in 1906. He went on to train in medicine in Heidelberg (where he no doubt would have been exposed to the then intractable problem of cancer), qualifying in 1911. Warburg remained in Heidelberg until the outbreak of the 1914–1918 war, although he occasionally visited the famous Marine Biological Station in Naples, where he performed experiments on the metabolism of fertilized sea urchin eggs that I will return to later. A key event occurred in 1913 when Warburg was 'head-hunted' to become a member of the Kaiser Wilhelm Gesellschaft at the instigation of Emil Fischer and Theodor Boveri (Boveri was the biologist who proposed, along with Walter Sutton, that chromosomes were the carriers of genetic inheritance, and made the far-sighted proposal that cancer might be caused by chromosomal re-arrangements). The Kaiser Wilhelm Gesellschaft comprised several elite scientific establishments spread over various disciplines and locations, whose members included luminaries such as Einstein, Haber, Hahn, Meitner and Polanyi. Describing the academic freedom available at these institutes to a prospective colleague, Warburg said 'You will be completely independent. No one will ever trouble you. No one will ever interfere. You may walk in the woods for a few years or, if you like, you may ponder over something beautiful'.

Unfortunately, before he could enjoy this enticing prospect, the First World War broke out. Warburg served in an elite Prussian cavalry regiment, reaching the rank of Lieutenant and being awarded the Iron Cross, First Class. In 1918, he was wounded and, while convalescing, received a letter from Albert Einstein beseeching him to leave the army and focus on his scientific career instead. This he did, perhaps because it was already becoming clear that Germany would be on the losing side. He took up his delayed appointment as Professor at the Kaiser Wilhelm Institute for Biology in Berlin-Dahlem, his location during the early 1920s where he did the experiments that led to the 'Warburg effect'. Despite the economic difficulties in post-war Germany, Warburg seems to have been reasonably well funded during this period. In a now legendary

research grant application that he made to the Emergency Association of German Science (which supported key scientific work immediately after the war), all he wrote was 'I need 10,000 Marks' – this very concise application was apparently successful! Nevertheless, according to his biographer Sir Hans Krebs, he only had space for about six people in the lab at this time, and much of the work was done by Warburg himself with the aid of some very skilled technicians and occasional visitors.

## Studies on the metabolism of cancer cells

One of the key questions driving Warburg's entire career was how living cells produce the energy that is used to power their growth and other functions. One of his earliest independent papers (Warburg 1908) described observations that when sea urchin eggs were fertilized, their rate of oxygen consumption increased up to 6-fold, presumably to provide energy for the very rapid cell divisions that subsequently ensued. Interestingly, Warburg's mentor Theodor Boveri used the fertilization of sea urchin eggs to study the behaviour of chromosomes during cell division, while many years later Tim Hunt used the same system to identify the cyclin proteins critical for cell division (Evans *et al.* 1983). Warburg may have guessed that, in terms of their metabolism, rapidly dividing tumour cells might behave in a similar manner to fertilized sea urchin eggs. However, he was not in a position to study this until he started his laboratory at Berlin-Dahlem after the war.

It should be remembered that the knowledge of metabolism at that time was much more rudimentary than it is today. As described in the next two sections, the catabolism of sugars was known to occur in two stages, i.e. fermentation (glycolysis) and respiration (oxidative metabolism). However, very little was known about what happened within each of those two stages.

## Fermentation

It had been known since ancient times that sugars in the extracts of malted barley or grape juice would 'ferment' into a liquid containing an intoxicant, although the chemical identification of the latter as ethanol was not made until the early 19th century. Fermentation also occurs during the making of leavened bread. In addition to the production of ethanol, a gas is given off vigorously during fermentation, creating the 'fizz' in beer and sparkling wine and the air pockets in bread (the word fermentation comes from

the Latin word ‘fervere’, to seethe or boil). These ancient discoveries led to the large-scale production of beer, wine and bread, arguably the first applications of biotechnology by humans.

The gas given off during fermentation to ethanol is carbon dioxide, first identified by the Scottish chemist Joseph Black in the 18th century. A deposit of ‘yeast’ was also known to accumulate during fermentation, and this had been shown to be a living organism, a type of fungus, by Theodor Schwann in 1837. However, the critical requirement for ‘beer yeast’ (*Saccharomyces cerevisiae*) for fermentation to occur was controversial and not fully accepted until the classic experiments of Louis Pasteur with swan-neck flasks in the 1850s, when he proved that sterilized sugar solutions would only ferment if exposed to yeast from the air. Pasteur also showed that another type of fermentation, generating lactic acid instead of ethanol, was caused by contaminating micro-organisms that spoiled wine (he called these ‘lactic yeast’, but they were most likely bacteria of the genus *Lactobacillus*). Fermentation of sugars to lactic acid, which is not accompanied by the evolution of carbon dioxide, had also been used by humans since ancient times to preserve foodstuffs, examples of such fermented products being yoghurt, sauerkraut and kimchi. Lactic acid was isolated from sour milk by Carl Wilhelm Scheele in 1780 and from extracts of mammalian muscle by another Swedish chemist, Jöns Jacob Berzelius, in 1808. However, it would be more than a century before it was proved that lactate in muscle was produced by a pathway similar to that involved in bacterial fermentation.

A landmark discovery in metabolism came in 1897 when Eduard Buchner showed that a cell-free extract of beer yeast would carry out fermentation of glucose to ethanol and carbon dioxide (described in his Nobel Prize lecture (Buchner 1907)). It can be argued that this finding represented the birth of the discipline of biochemistry because it allowed subsequent fractionation of the extract into its individual components. However, it was not until around 1940 that Otto Meyerhof and others had established the full pathway of what we now call glycolysis. Therefore, at the time of Warburg’s study of cancer metabolism in the 1920s, glycolysis was essentially a black box where glucose fed in at one end and lactate emerged at the other.

## Respiration

The necessity for continued breathing to sustain life in animals must have been self-evident to ancient humans. By the 18th century, it was known that animals breathed in one type of air (containing *oxygen*, identified and named by

Antoine-Laurent de Lavoisier) and breathed out a different type containing *carbon dioxide* (identified and named ‘fixed air’ by Joseph Black, and identical with the gas given off during fermentation to ethanol, see earlier). Lavoisier also correctly identified the analogies between respiration and combustion, writing in 1789 that: ‘respiration is nothing but a slow combustion of carbon and hydrogen, which is entirely similar to that which occurs in a lamp or candle, and that, from this point of view, animals that respire are true combustible bodies that burn and consume themselves’. Lavoisier is acknowledged to be one of the fathers of modern chemistry, but, unfortunately for him, he was also an aristocrat involved in collecting taxes prior to the French Revolution, and his prowess in science did not prevent him from being sent to the guillotine in 1794 after the revolution broke out.

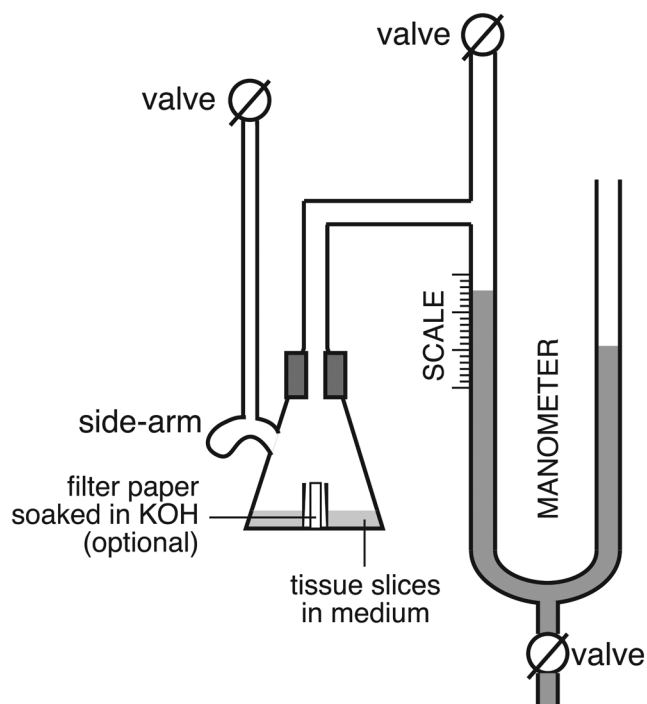
In terms of detailed mechanism, respiration was an even tougher nut to crack than fermentation, mainly because it requires intact mitochondria to function. Indeed, it was not until just before this author began to study biochemistry in the late 1960s that the final link was established with the chemiosmotic hypothesis of Peter Mitchell, in which he proposed (correctly) that the respiratory chain created a gradient of  $H^+$  ions across the mitochondrial inner membrane, which was then used to drive ATP synthesis (described in his Nobel lecture (Mitchell 1978)). The Nobel prize awarded to Warburg himself in 1931 was not in fact for his work on what we now call the Warburg effect but for his realization that respiration involves the oxidation/reduction of  $Fe^{2+}/Fe^{3+}$  ions. This was published (Warburg 1925a) just after he had performed his initial studies on cancer cell metabolism.

## Techniques available to Warburg in the 1920s

Thus, at the beginning of the 1920s when Warburg started his own lab at the Kaiser Wilhelm Institute in Berlin-Dahlem, he knew that the metabolism of sugars by animal tissues occurred in two stages, i.e. fermentation or glycolysis, in which glucose or other sugars were broken down into lactate, and respiration or oxidative metabolism, in which sugars were fully oxidized to carbon dioxide. However, little was known about the intermediate steps in either of these pathways. Warburg’s goal was to determine how living cells generated the energy required for their growth and proliferation, and to study this, he had two main methods at his disposal, the Warburg manometer to measure gas exchange and studies of metabolism using tissue slices.

### The Warburg manometer

This instrument was the 1920s equivalent of the extracellular flux analysers that we use today to measure oxygen consumption rate. Although it is now usually named after Warburg, in fact, it was derived from the manometer used to monitor the exchange of blood gases by the English physiologists Haldane and Barcroft. It comprised (Fig. 1) a conical flask in which tissues were incubated in a liquid medium, which was connected via a ground glass joint to the manometer itself. The conical flask had a central well which could contain a filter paper soaked in alkaline solution to absorb carbon dioxide and a side arm that allowed the addition of solutions to the flask during the experiment. The manometer was a U-shaped glass tube containing sodium chloride solution (being less dense than the mercury used in meteorological pressure gauges, this was more sensitive to small pressure changes). The instrument was designed so that the gas phase that connected the sample and the manometer had an essentially constant volume, and the conical flask was shaken in a water bath at a constant temperature. If gases were evolved or absorbed by the sample, this, therefore,



**Figure 1**

Schematic diagram of a Warburg manometer. Most of the apparatus would have been immersed in a shaking water bath to maintain a constant temperature and mixing of the medium. The manometer fluid would have risen up the left-hand arm of the manometer as oxygen was consumed, while the volume of enclosed air would not have changed significantly. Based on equipment depicted in [Lighton \(2008\)](#).

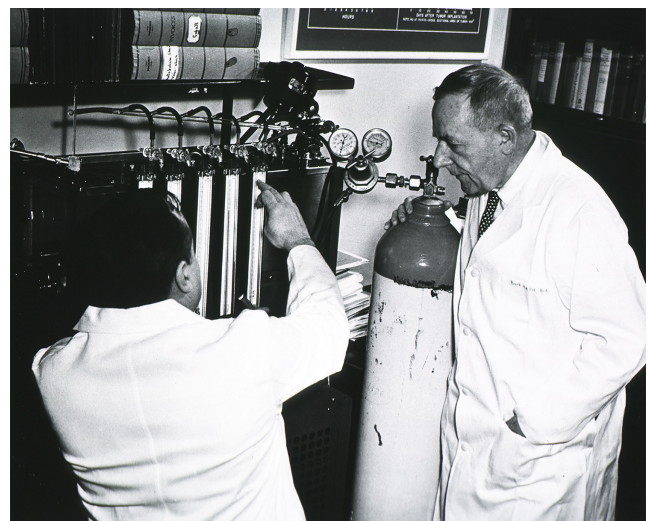
created a change in pressure that could be measured by differences in the height of the fluid in the two arms of the manometer.

The Warburg manometer also allowed measurement of the production of lactic acid. If a solution of bicarbonate was added to the flask via the side arm, carbon dioxide was produced in proportion to the amount of lactic acid in the medium. This was the equivalent of measuring the extracellular acidification rate using modern extracellular flux analysers.

The Warburg manometer required great care in its set-up and operation and to be carefully calibrated. I know this to my cost because when I started second-year biochemistry as an undergraduate in 1969, the first 2 weeks involved measuring the gas exchange of tissues using this instrument. I suspect that if Warburg (who died in 1970) had been watching my efforts (see [Fig. 2](#)), he would not have offered me a job!

### Tissue slices

The other innovation developed by Warburg was the use of slices of tissue to study metabolism. He realized that these needed to be thin enough to allow adequate rates of exchange of nutrients and gases between the tissue and the medium, but not so thin that the results would be compromised by products of cells at the periphery that were damaged when the slices were cut.



**Figure 2**

Otto Warburg (right) discussing a manometer experiment with an assistant (Public domain image from the National Library of Medicine digital collection, <http://resource.nlm.nih.gov/101442335>).



## Initial studies by Warburg on metabolism of tumour cells

Warburg started his studies using slices of the Flexner–Jobling carcinoma (a tumour of rat seminal vesicles that was transplantable in rats, developed at the Rockefeller Institute in 1906). He compared slices of these with normal rat liver or kidney as controls, all incubated in Ringer's medium. His expectation was that, like the fertilized sea urchin eggs, there would be a large increase in respiration in the cancer cells, but, to his great surprise, respiration was actually considerably less than that observed in the control tissues. Since Ringer's solution contains no carbon sources, he tried adding glucose, fatty acids or amino acids to see whether the tumour slices were deficient in nutrients, but the only effect of this was that the addition of glucose depressed respiration even further. Warburg eventually realized the explanation was that the tumour tissue was producing a considerable amount of lactate. He estimated, using a nitrogen atmosphere in the absence of oxygen, that tumour slices: 'produced  $\approx 100$  times as much lactic acid as does blood,  $\approx 200$  times as much as frogs' muscle at rest, and 8 times as much as frogs' muscle working to the limit of its normal efficiency' (Warburg 1925b). When oxygen was reintroduced to tumour slices, he found that there was some decline in glycolysis, but nothing like as large as in the control tissues. He stated (Warburg 1925b): 'We regard it as the most important of our findings, that in its metabolism carcinoma tissue does not behave like muscle or *Mucor mucedo*, but like yeast'. Here, 'yeast' refers to beer yeast (*Saccharomyces cerevisiae*), while *M. mucedo* is pin-mould, another fungus that had been studied by Pasteur. Thus, when oxygen was reintroduced to normal tissue slices or *M. mucedo*, the rate of glycolysis would decline rapidly with no lactate then being produced, whereas this was much less marked in tumour tissue or in proliferating *S. cerevisiae*. He quantified this by estimating the ratio of lactate consumed or produced to oxygen taken up when the latter was reintroduced. For normal tissues, such as muscle, around one or two molecules of lactate were *consumed* for every molecule of oxygen used. In marked contrast, in the Flexner–Jobling carcinoma, around four molecules of lactate were *produced* for every molecule of oxygen consumed. To put this in context, Warburg (1925b) argued 'One molecule of lactic acid means the splitting up of one molecule of glucose, one molecule of respired oxygen the oxidation of one sixth of a molecule of glucose; from which it is evident that of thirteen molecules of glucose which the tumor attacks, it oxidizes one molecule and splits up the remaining twelve. The metabolism of carcinoma tissue in

oxygen is, then, preponderantly a splitting metabolism'. While we know now that the splitting of one molecule of glucose during glycolysis gives rise to *two* molecules of lactate rather than one, the fact remains that Warburg had discovered that tumour tissue is highly glycolytic. This was not just confined to the Flexner–Jobling rat carcinoma, because Warburg surveyed a range of human tumours and found they had lactate:oxygen ratios of 3.0–3.5, similar to the value of around 4 for the rat carcinoma. Benign tumours such as bladder papillomas and nasal polyps also continued to produce lactate in the presence of oxygen, but their lactate:oxygen ratios were much lower, at around one. Thus, 'malignant tumors produce three to four times more lactic acid per molecule of oxygen consumed than do benign tumors' (Warburg 1925b).

Warburg did realize that the tumours he was studying were a mixture of normal and cancer cells, which might have affected the results. Many years later he studied ascites cells, for which it was possible to obtain an almost pure preparation of cancer cells, and found that their ratio of lactate produced to oxygen consumed was about 8 (Warburg 1956), around twice as high as in the solid tumours he had studied.

One interesting exception to the rule that high rates of glycolysis are not seen in quiescent tissues, discovered by Warburg himself, was the retina of the eye. Under anaerobic conditions, he found that rat retina produced lactate at a higher rate than many tumours – when oxygen was reintroduced, the production of lactate dropped but did not disappear entirely, as it did in most quiescent tissues. This led him to state (Warburg 1925b): 'The case of the retina obviously makes it necessary to proceed with caution in dogmatizing from our results. We may say for the higher organisms: no growth without glycolysis; but not conversely, no glycolysis without growth'. I will return to this interesting exception to the Warburg hypothesis at the end of this article.

## Contemporary reaction to Warburg's findings

It is hard to judge what impact (if any) Warburg's 1923 papers had on oncologists at the time, but they were clearly noticed by other biochemists, although the latter were fewer in number than they are today. His findings were, for example, rapidly followed up by the husband and wife team of Carl and Gerti Cori, who were later (1947) to win the Nobel prize in Physiology and Medicine for their work on glycogen metabolism. A deficiency in Warburg's studies, which he himself recognized, was that all of the

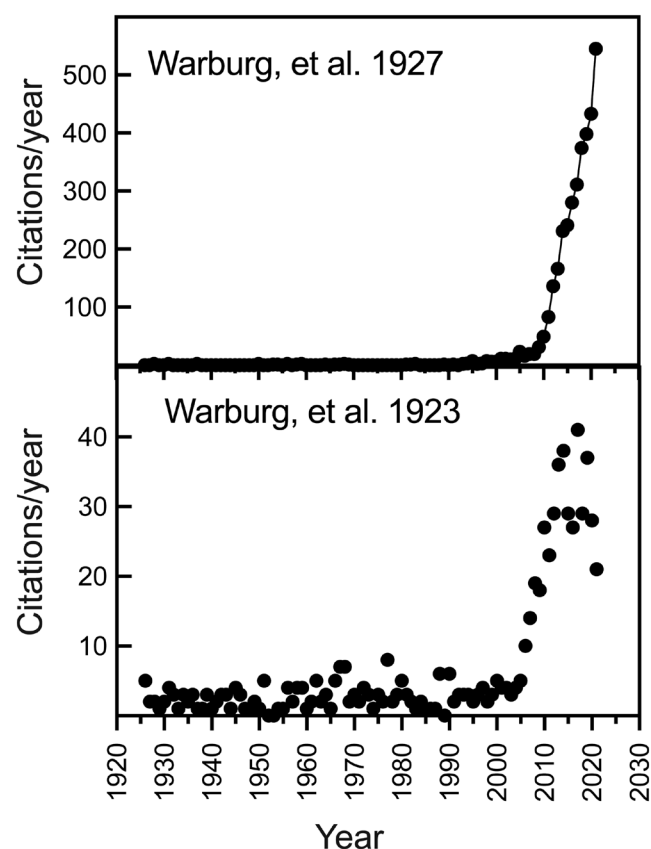
measurements had been made with tissue slices *in vitro*, with no analyses *in vivo*. The Cori's addressed this in two papers. In the first (Cori & Cori 1925b), they dissected out tumours from mice and rats and measured their contents of free glucose and lactate by chemical methods. Consistent with the Warburg hypothesis, the tumours did appear to have somewhat lower content of free glucose and a higher content of lactate than normal tissues, although they admitted that the results might have been compromised by glucose and lactate being washed out during dissection of the tumours. More convincing was the second paper (Cori & Cori 1925a), where they studied a chicken sarcoma, originally caused by the Rous sarcoma virus (to be discussed later), but for which tumours were transplantable in chickens. Tumour cells were implanted into the musculature of one wing, leaving the other as a control, and once the tumours were established, blood was drawn from the wing veins on either side. The results clearly showed that the glucose and CO<sub>2</sub> were lower, and lactate content was higher, in venous blood on the tumour side. They also studied a single human patient with a large sarcoma on one forearm and also found lower glucose and higher lactate content in venous blood from that arm. These results, therefore, provided some support that Warburg's findings were also relevant *in vivo*.

## The next 80 years

What happened to Warburg's findings during the next 80 years? The answer seems to be, not much. The term 'Warburg effect' appears to have been first used by Efraim Racker (Racker 1972), and it was used as part of the title ('Warburg Effect Revisited') of a paper published in *Science* (Racker & Spector 1981) in which a cascade of protein tyrosine kinases, the most upstream of which was the oncogene product Src (see the next section), was claimed to phosphorylate the plasma membrane Na<sup>+</sup>/K<sup>+</sup> pump. This was in turn proposed to uncouple the ATPase activity of the pump from ion transport, causing turnover of ATP to ADP, with the availability of the latter then accelerating glycolysis and hence the Warburg effect. Unfortunately, for Racker, who was highly respected for having isolated the F1 component of the mitochondrial ATP synthase in 1960, it turned out that his assistant Mark Spector had fabricated many of the results (Broad & Wade 1985), and the paper was quickly withdrawn (Racker 1981). Ironically, the withdrawn paper (Racker & Spector 1981) had actually started with a quotation from G.K. Chesterton: 'There are no rules for architecture for a castle in the clouds'. This

unfortunate episode may have tarnished the concept of the Warburg effect, at least temporarily.

The original 1923 papers by Warburg have never been particularly highly cited, most likely because they were published in German. Figure 3 shows citation analysis for one of the 1923 papers published in the *Biochemische Zeitschrift* (Warburg *et al.* 1923) and for a more highly cited follow-up paper that was published in English in the *Journal of General Physiology* (Warburg *et al.* 1927). Up to the mid-1990s, neither paper received more than 10 citations per year, but things really took off in the late 2000s, with more than 500 citations to the 1927 paper in 2021 alone. This remarkable distribution shows that Warburg's work on cancer was well ahead of its time, and following the initial publication went into a state of dormancy that was to last around 80–90 years. Why was this? Was there perhaps simply not enough known about metabolism in the 1920s to take things any further? As already indicated, the pathway of glycolysis was not fully worked out until the 1940s, and that of respiration not until the late 1960s,



**Figure 3**

Citation analysis of one of Warburg's papers from 1923 published in German (Warburg *et al.* 1923), and a follow-up paper published in English (Warburg *et al.* 1927). Citation counts were obtained from Google Scholar for every year subsequent to publication.

culminating in the chemiosmotic hypothesis of Mitchell and the proposal by Lynn Margulis (then known by her married name, Sagan) that mitochondria may have been derived by endosymbiosis of oxidative bacteria (Sagan 1967). After the 1960s, studies of metabolism then appear to have fallen out of fashion, probably because attention became focused on the exciting discoveries being made on DNA, the genetic code and molecular genetics.

### The 1970s to 2000 – discovery of oncogenes and tumour suppressors

During the decades from the 1920s to the 1960s, the treatment of cancer had made progress, based on the use of surgery and radiotherapy, and of chemotherapy using a wide range of toxic compounds with severe side effects (Mukherjee 2011). Nevertheless, these interventions had largely been designed by trial and error, and the underlying molecular basis of cancer had remained stubbornly unclear. Things began to change in the 1970s with the development of DNA cloning and sequencing technology. There was a gradual realization that cancer was usually caused by somatic mutations in a relatively limited number of critical genes involved in cellular growth control, which we now refer to as oncogenes and tumour suppressors. In 1914, one of Warburg's mentors, Theodor Boveri, had made the far-sighted suggestion that cancer might be caused by chromosomal rearrangements. However, even before that Peyton Rous (1910) had identified a transmissible sarcoma in chickens that was later shown to be caused by a virus, the Rous sarcoma virus (RSV). This led many cancer researchers (even up to the 1970s (Mukherjee 2011)) to favour the idea that human cancer was also usually caused by viruses, i.e. by external infectious agents, rather than by internal processes such as chromosomal re-arrangements. In fact, RSV was involved in a key breakthrough when it was realized that, compared with related viral strains that did not cause sarcomas, RSV contained an extra gene (v-Src) that was closely related to a gene in the normal chicken host (Spector *et al.* 1978). The virus had probably picked up this gene accidentally during its life cycle as an RNA virus or *retrovirus*, which involves copying the RNA genome into DNA that is then inserted into the host genome before being copied back into RNA again. The extra gene turned out to encode a protein kinase that phosphorylated tyrosine residues (Collett *et al.* 1980), but sequencing also revealed that the viral protein (v-Src) had lost some C-terminal residues that normally restrain the activity of the endogenous chicken protein (c-Src),

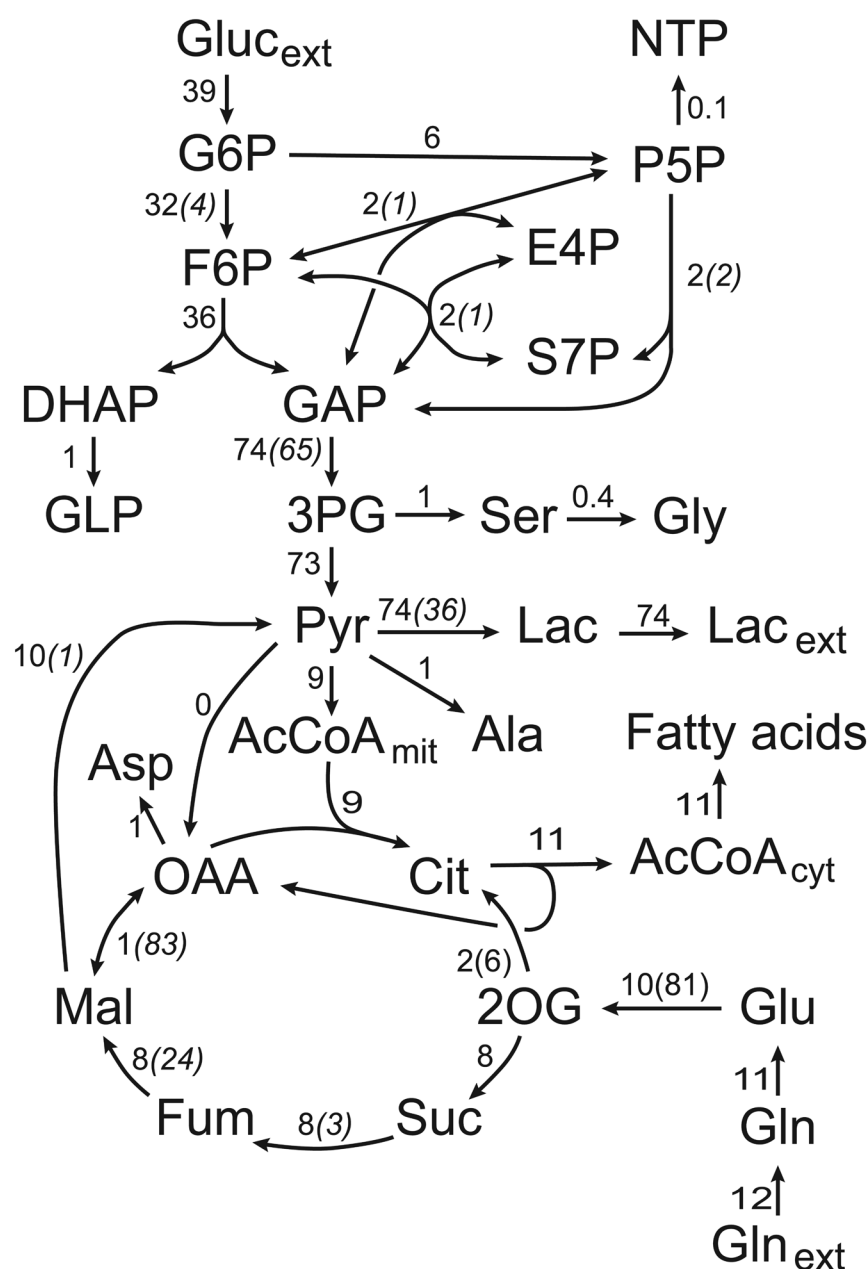
rendering v-Src hyperactive (Takeya & Hanafusa 1983). Although we now believe that the vast majority of human cancers are caused not by viruses but by somatic mutations triggered by mutagens such as ultraviolet light or cigarette smoke, v-Src nevertheless established a paradigm that explained how proto-oncogenes (e.g. *c-Src*) could be converted into oncogenes (e.g. *v-Src*) by mutations that rendered the encoded protein constitutively active and no longer responsive to control by upstream activators, such as growth factors. Numerous other oncoproteins were subsequently discovered (Mukherjee 2011), including activated mutants of the Ras family of G proteins, of growth factor receptors such as *HER2/ERBB2*, and of signalling proteins downstream of growth factor receptors such as PI-3-kinases (e.g. *PIK3CA*). A number of tumour suppressors were also identified, including phosphatase and tensin homologue (PTEN), which normally restrains cell growth by breaking down phosphatidylinositol-3,4,5-trisphosphate (Maehama & Dixon 1998), the product of PI-3-kinases and a key second messenger that activates the protein kinase Akt. With a few notable exceptions discovered later (Baysal *et al.* 2000, Tomlinson *et al.* 2002, Gottlieb & Tomlinson 2005, Parsons *et al.* 2008), the majority of proto-oncogenes and tumour suppressors were proteins involved in cell signalling and cellular growth control, rather than metabolism. This may have focused minds during the 1980s and 1990s on the former rather than the latter.

### The 2000s – rediscovery of the Warburg hypothesis

What caused the renewed interest in the Warburg hypothesis in the new millennium, which resulted in the sudden increase in citations to his early papers (Fig. 3)? One trigger appears to have been the development of 2-[F<sup>18</sup>] fluoro-2-deoxyglucose-positron emission tomography (FDG-PET) as a technique to image solid tumours in humans. FDG is a glucose analogue that is taken up into cells via glucose transporters but not metabolized beyond FDG-6-phosphate; it therefore accumulates in cells that have a high rate of glucose uptake. FDG-PET was found to be effective in imaging solid tumours that have a high rate of glucose uptake, which is of course a corollary of the Warburg effect, and papers discussing FDG-PET appear to have begun citing Warburg around 80 years later (e.g. Bos *et al.* 2002). A second trigger may have been the discoveries, already cited earlier, that loss-of-function mutations in genes encoding metabolic enzymes, such

as the tricarboxylic acid (TCA) cycle enzymes succinate dehydrogenase or fumarate hydratase, could in rare cases cause tumours such as paragangliomas, uterine fibroids, leiomyomata or renal cell carcinomas (Baysal *et al.* 2000, Tomlinson *et al.* 2002). A third trigger was the development in the 2000s of techniques of metabolomics, whereby the levels of many cellular metabolites could be estimated simultaneously by mass spectrometry and gas chromatography or nuclear magnetic resonance, and where precursors such as glucose labelled with the stable isotope  $^{13}\text{C}$  could be used to estimate relative fluxes through different metabolic pathways. I well remember the first

paper that I came across that used metabolic flux analysis (Metallo *et al.* 2009), which caught my eye because it utilized A549 cells, a human lung adenocarcinoma cell line that I knew had loss-of-function of the tumour suppressor LKB1 and would therefore have low AMP-activated protein kinase (AMPK) activity (see later). The paper suggested that for every molecule of glucose taken up, about 90% of the flux (74 nmol/min/mg protein) went to the secretion of lactate, with only about 10% (9 nmol/min/mg) entering the TCA cycle, in excellent agreement with the Warburg effect (Fig. 4). Even more remarkably, the flux from citrate in the TCA cycle to cytoplasmic acetyl-CoA for fatty acid



**Figure 4**

Results of metabolic flux analysis (Metallo *et al.* 2009) in A549 cells grown in DMEM medium with 10% fetal bovine serum, 25 mM glucose and 4 mM glutamine. Numbers in each arrow are estimates of fluxes in nmol.min<sup>-1</sup>.mg protein<sup>-1</sup>; net fluxes are listed first, while those in parentheses are exchange fluxes. Figures in italics are estimates from the literature, when exchange fluxes could not be determined with sufficient precision in this study. 2OG, 2-oxoglutarate; 3PG, 3-phosphoglycerate; AcCoA<sub>cyt/mit</sub>, acetyl-CoA (cytosolic/mitochondrial); Ala, alanine; Asp, aspartate; Cit, citrate; DHAP, dihydroxyacetone phosphate; E4P, erythrose-4-phosphate; F6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; Gln/Glu<sub>ext</sub>, glutamine/extracellular glutamine; GLP, glycerol-3-phosphate; Glu<sub>ext</sub>, extracellular glucose; Gly, glycine; Lac/Lac<sub>ext</sub>, lactate/extracellular lactate; Mal, malate; NTP, nucleoside triphosphate; OAA, oxaloacetate; P5P, pentose-5-phosphate; Pyr, pyruvate; S7P, sedoheptulose-7-phosphate; Ser, serine; Suc, succinate. Adapted, from *Journal of Biotechnology*, Vol 144, Metallo CM, Walther JL & Stephanopoulos G, Evaluation of  $^{13}\text{C}$  isotopic tracers for metabolic flux analysis in mammalian cells, Pages 167–174, Copyright (2009), with permission from Elsevier.



synthesis via ATP-citrate lyase ( $\text{Cit} \rightarrow \text{AcCoA}_{\text{cyt}} + \text{OAA}$ ; 11 nmol/min/mg) was actually slightly greater than that of acetyl-CoA entering the cycle in the first place (9 nmol/min/mg). This was only possible because carbon from glutamine (Gln) was entering the cycle to restore the levels of 2-oxoglutarate (2-OG) (bottom right of Fig. 4), via a so-called *anaplerotic* pathway, i.e. one that replenishes the levels of pathway intermediates. The TCA cycle was effectively no longer operating as a cycle, but rather as a branched pathway that diverged at 2-OG, with part of the flux going 'forward' to succinate, fumarate and malate, and part going 'backward' to citrate (Fig. 4). The backward flux from 2-OG to citrate, termed reductive carboxylation, was later shown to occur in other proliferating cells, and to use the cytosolic, NADP<sup>+</sup>-linked IDH1 isoform of isocitrate dehydrogenase, rather than the mitochondrial, NAD<sup>+</sup>-dependent IDH2 isoform (Metallo *et al.* 2011, Mullen *et al.* 2011). Note that Hans Krebs used pigeon breast muscle, a quiescent tissue, to work out his famous cycle – things might have been different if he had been using cultured tumour cells! Another interesting feature of Fig. 4 is the high flux from malate to pyruvate via malic enzyme ( $\text{Mal} \rightarrow \text{Pyr}$ ; 10 nmol/min/mg). Along with the oxidative branch of the pentose phosphate pathway ( $\text{G6P} \rightarrow \text{P5P}$ ), this would provide NADPH that could be used in fatty acid synthesis; malic enzyme also helps to recycle carbon atoms from oxaloacetate (OAA), generated in the ATP-citrate lyase reaction, back into fatty acid synthesis.

It is important to note that the estimates of fluxes shown in Fig. 4 required mathematical modelling with several assumptions necessary for their derivation (Metallo *et al.* 2009), and the estimates would almost certainly be different in different cells and under different growth conditions. Indeed, there is evidence that *Kras* mutant lung cancer cells (similar to A549 cells) are much less reliant on glutamine when grown *in vivo* rather than in culture and appear to use glucose metabolism via pyruvate carboxylase, rather than glutamine, for anaplerosis of TCA cycle intermediates (Davidson *et al.* 2016) (note that in Fig. 4 the flux through pyruvate carboxylase ( $\text{Pyr} \rightarrow \text{OAA}$ ) is negligible). Despite these caveats, the results shown in Fig. 4 were a revelation, particularly with respect to the quantitative importance of fatty acid synthesis in the metabolism of rapidly proliferating cells.

My own entry into the field of cancer and metabolism occurred in 2003, when we and others (Hawley *et al.* 2003, Woods *et al.* 2003) reported that LKB1 was the critical upstream kinase required for the activation of AMPK when cellular energy levels were falling. AMPK had been discovered as a protein kinase that inactivated fatty acid

synthesis by phosphorylating acetyl-CoA carboxylase (Carlson & Kim 1973) but had subsequently been shown, when activated by cellular energy stress, to switch on several catabolic processes while switching off almost all anabolic pathways involved in cell growth, including synthesis of lipids, proteins, glycogen, ribosomal RNA and nucleotides (Gonzalez *et al.* 2020). LKB1, on the other hand, had been identified as a putative protein kinase that was the product of the *STK11* gene, in which heterozygous loss-of-function mutations in the germline were known to cause Peutz-Jeghers syndrome. The latter is a rare inherited predisposition to cancer in humans, but the *STK11* gene also undergoes relatively frequent somatic mutations in many sporadic cancers, especially in adenocarcinoma of the lung where it occurs in up to 20% of cases (Sanchez-Cespedes *et al.* 2002, Ji *et al.* 2007). Our findings therefore indicated a key link between LKB1, a tumour suppressor, and the metabolic controller AMPK.

A seminal review that helped to revitalize the Warburg effect and studies on cancer cell metabolism at the end of the 2000s was written by Vander Heiden *et al.* (2009). In it, they pointed out that while unicellular organisms tend to proliferate as long as nutrients are available, cells in most multicellular eukaryotes are bathed in a rich nutrient medium yet they only take up and utilize those nutrients for biosynthesis when 'given permission' to do so, typically by growth factors. However, cancer cells have undergone mutations that cause them to ignore some of these extracellular controls; they can be regarded as rebels that are no longer obeying orders from above and have reverted to a kind of ancestral unicellular lifestyle.

What are the causes for the rapid glucose utilization and lactate production in tumour cells and other rapidly dividing cells? Warburg himself came to believe that it was because cancer cells have defects in mitochondrial function (Warburg 1956), but that has not been supported by most subsequent evidence. A number of other reasons and mechanisms have been proposed – their relative importance is still the subject of debate and is likely to depend on the individual cell type and the exact growth conditions:

1. As mentioned earlier during the discussion of Fig. 4, these metabolic changes may be adaptations to allow rapid increases in biomass, particularly for the production of membrane lipids.
2. What normally limits glucose supply for metabolism is not its availability in the bloodstream, but the rate at which cells take it up via glucose transporters. If the latter increases, e.g. due to activation of the insulin signalling pathway, or oncogenic mutations

that activate downstream components of it, such as the protein kinase Akt, increased glycolysis might maintain an increased cellular energy status that would keep AMPK activity low. In addition, the  $\alpha 1$  isoform of AMPK is directly phosphorylated by Akt, which inhibits its phosphorylation and activation by LKB1 (Hawley *et al.* 2014). Both mechanisms would lessen the restraints on biosynthetic pathways that would otherwise be mediated by AMPK.

3. The AMPK pathway is also mutually antagonistic with the mechanistic target-of-rapamycin complex-1 (mTORC1) pathway, which is activated by nutrient availability and promotes cell growth (Gonzalez *et al.* 2020, Ling *et al.* 2020). Interestingly, while AMPK inhibits fatty acid synthesis and promotes oxidative metabolism (the latter by increasing mitochondrial biogenesis and recycling of damaged mitochondria by mitochondrial fission and mitophagy (Gonzalez *et al.* 2020)), mTORC1 promotes glycolysis by increasing expression of the transcription factor hypoxia-inducible factor-1 $\alpha$  (HIF1A) and promotes the pentose phosphate pathway and fatty acid synthesis by increasing expression of the transcription factors, SREBP1/SREBP2 (Duvel *et al.* 2010). Thus, while mTORC1 would promote the Warburg effect, AMPK opposes it.
4. The pentose phosphate pathway, which can be an alternative to the first part of glycolysis, generates NADPH required for lipid biosynthesis and repair of oxidative damage. If the pentose phosphate shunt is followed by complete oxidation to CO<sub>2</sub>, a single molecule of glucose can generate around 30 molecules of ATP plus 2 molecules of NADPH. By contrast, if it is used with subsequent metabolism only as far as lactate, one molecule of glucose generates less than two molecules of ATP plus two molecules of NADPH. Since one molecule of ATP and two of NADPH are required for each two-carbon unit added during fatty acid synthesis, the production of ATP and NADPH are much better balanced for the purposes of fatty acid synthesis if glucose metabolism terminates at lactate. In addition, in cells with a high demand for fatty acids, a large part of any flux that does enter the TCA cycle via citrate synthase (OAA + AcCoA<sub>mit</sub> → Cit) is likely to immediately leave the cycle again, since citrate is exported to the cytoplasm and converted by ATP-citrate lyase into cytosolic acetyl-CoA (Fig. 4), one molecule of which is required for every two-carbon unit added during fatty acid synthesis. This proportion of citrate would not be available for ATP

production by oxidative metabolism, thus reducing oxygen consumption.

5. The pentose phosphate pathway can also be used to generate ribose-5-phosphate used in nucleotide biosynthesis. However, note that in proliferating A549 cells, the flux to nucleotides from the pentose phosphate pathway (P5P → NTP, Fig. 4) is trivial compared with that to fatty acids, despite the fact that the medium used (DMEM) was not supplemented with nucleosides.
6. In addition to the pentose phosphate pathway, two other anabolic pathways branch from glycolysis, i.e. synthesis of glycerol-3-phosphate used in glycerolipid synthesis (DHAP → GLP), and the synthesis of serine (3PG → Ser) which then, via tetrahydrofolate derivatives, donates one-carbon units during the synthesis of nucleotides and some amino acids. Perhaps the Warburg effect is in part a reflection of the flux out of glycolysis into these alternate pathways? While this is an attractive idea, the flux into these pathways, at least in A549 cells grown in DMEM, is only a very small fraction of the total flux through glycolysis (Fig. 4). Also, while flux into these pathways could help to explain the high glucose uptake of tumour cells, they would not explain the high lactate output.
7. One possible further benefit of the Warburg effect is decreased oxygen consumption, which would reduce the risk of necrosis due to the blood supply being inadequate until neovascularization of the tumour had occurred. However, this is unlikely to be a major factor, because cultured tumour cells display the Warburg effect even when saturating levels of oxygen are supplied (e.g. Fig. 4).

### Exceptions to the Warburg effect – sea urchin embryos and retinal photoreceptor cells

I will finish this article by discussing two exceptions to the Warburg effect that the man himself uncovered:

1. In the rapid cell divisions that occur in sea urchin eggs immediately after fertilization with sperm, Warburg found that there was a large increase in respiration without any increase in lactate production (Warburg 1908) – why do these cells behave differently to tumour cells and other rapidly proliferating cells? A key factor may be that the first few divisions of the fertilized sea urchin embryo, up to at least the 128-cell stage, occur

by very rapid cleavages with no increase in biomass, i.e. no growth. The cells, therefore, simply get smaller, using nutrients stored in the egg to generate the ATP required for their very rapid divisions. Since these nutrient stores are finite, it would make sense that the much more energy-efficient oxidative metabolism should be utilized. This emphasizes the fact that the Warburg effect is really a feature of *growing* rather than *dividing cells*.

2. Warburg also discovered that cells in the retina of the eye produced large quantities of lactate even though they are post-mitotic and quiescent (Warburg 1925b) – why? Some interesting proposals to explain this have been made (Leveillard & Sahel 2017, Viegas & Neuhauss 2021). The retina ranks amongst the highest of all tissues in terms of energy consumption. Its photoreceptor cell layer consists of about 95% rods, used for monochrome vision in dim light, and 5% cones, used for colour vision in bright light. The photoreceptor in both is *rhodopsin*, comprising variants of the protein opsin in complex with the chromophore retinal; the absorption of a photon of light causes a switch from 11-*cis*- to all-*trans*-retinal, triggering a change in opsin conformation. Opsin is a typical G-protein-coupled receptor with seven transmembrane domains, which in rods is embedded at very high abundance in numerous parallel stacks of membrane in the rod outer segment. In order for the conformational change that occurs upon light absorption to be detected, it is necessary for the membranes in these stacks to be very fluid, so they contain many polyunsaturated fatty acids (PUFAs) including docosahexaenoic acid (22:6, n-3). However, PUFAs are particularly susceptible to oxidative damage induced by light absorption, and perhaps for that reason as much as 10% of the rod outer segment is removed every day by phagocytosis by the retinal pigmented epithelial (RPE) cells that abut against the outer end of the rod outer segments. The lipids and proteins in the engulfed membrane stacks will have to be replaced by new membrane stacks that are synthesized at the opposite end of the rod outer segment and gradually move down to replace the stacks that have been phagocytosed. The rod cells therefore must have a very high capacity to synthesize new membrane lipids and proteins to replace the large amount of biomass lost each day. Thus, although the cells are not dividing, they are nevertheless growing rapidly, which may be why they display the Warburg effect. Another likely reason is that NADPH generated

in the pentose phosphate pathway is required to maintain reduced glutathione and thioredoxin to repair the extensive oxidative damage that occurs to both lipids and proteins on exposure to light.

## Conclusions

The citation analysis shown in Fig. 3 confirms that Warburg's papers on tumour cell metabolism published in the 1920s (Warburg 1923, 1925b, Warburg & Minami 1923, Warburg *et al.* 1923, 1924) were at least 80–90 years ahead of their time. It took another 40 years to fill in the gaps in our knowledge of metabolism, and then in the 1970s, basic studies of cancer biology shifted towards molecular genetics and the identification of oncogenes and tumour suppressor genes. It was only with the development of FDG-PET as a technique for imaging tumours towards the end of the 20th century, and of metabolomics and metabolic flux analysis at the start of the 21st, that Warburg's seminal findings were rediscovered and had a considerable impact. As to the molecular mechanisms that underlie the Warburg effect, although there have been many suggestions, this remains incompletely understood. One possibility is that some of the metabolic changes might be explained by changes in the concentrations of the classical allosteric regulators of metabolic enzymes, which were mainly identified in the 1950s and 1960s and are described in biochemistry textbooks, even though in most cases it has not been shown that these controls actually operate in intact mammalian cells. With the techniques available today, it is possible to study this by making cells or whole animals with knock-in mutations that render the metabolic enzymes insensitive to these allosteric regulators while still catalytically active. With some notable exceptions (Bouskila *et al.* 2010, Hunter *et al.* 2018), this approach, which first requires the identification of mutants insensitive to the allosteric regulators, has not yet been widely used.

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The author declares that there is no conflict of interest that may be perceived as prejudicing the impartiality of this review.

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## References

- Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, *et al.* 2000 Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* **287** 848–851. (<https://doi.org/10.1126/science.287.5454.848>)
- Bos R, van Der Hoeven JJ, van Der Wall E, van Der Groep P, van Diest PJ, Comans EF, Joshi U, Semenza GL, Hoekstra OS, Lammertsma AA, *et al.* 2002 Biologic correlates of (18)fluorodeoxyglucose uptake in human breast cancer measured by positron emission tomography. *Journal of Clinical Oncology* **20** 379–387. (<https://doi.org/10.1200/JCO.2002.20.2.379>)
- Bouskila M, Hunter RW, Ibrahim AF, Delattre L, Pegg M, van Diepen JA, Voshol PJ, Jensen J & Sakamoto K 2010 Allosteric regulation of glycogen synthase controls glycogen synthesis in muscle. *Cell Metabolism* **12** 456–466. (<https://doi.org/10.1016/j.cmet.2010.10.006>)
- Broad W & Wade N 1985 *Betrayers of the Truth: Fraud and Deceit in Science*. Oxford, UK: Oxford University Press.
- Buchner E 1907 *Nobel Prize Lecture: Cell-free fermentation*. Stockholm, Sweden: Nobel Prize Outreach AB. (available at: <https://www.nobelprize.org/prizes/chemistry/1907/buchner/lecture/>)
- Carlson CA & Kim KH 1973 Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *Journal of Biological Chemistry* **248** 378–380. ([https://doi.org/10.1016/S0021-9258\(19\)44486-4](https://doi.org/10.1016/S0021-9258(19)44486-4))
- Collett MS, Purchio AF & Erikson RL 1980 Avian sarcoma virus-transforming protein, pp60src shows protein kinase activity specific for tyrosine. *Nature* **285** 167–169. (<https://doi.org/10.1038/285167a0>)
- Cori CF & Cori GT 1925a The carbohydrate metabolism of tumors. II. Changes in the sugar, lactic acid, and combining power of blood passing through a tumor. *Journal of Biological Chemistry* **65** 397–405.
- Cori CF & Cori GT 1925b The carbohydrate metabolism of tumors. I. The free sugar, lactic acid and glycogen content of malignant tumors. *Journal of Biological Chemistry* **64** 11–22. ([https://doi.org/10.1016/S0021-9258\(18\)84944-4](https://doi.org/10.1016/S0021-9258(18)84944-4))
- Davidson SM, Papagiannakopoulos T, Olenchock BA, Heyman JE, Keibler MA, Luengo A, Bauer MR, Jha AK, O'Brien JP, Pierce KA, *et al.* 2016 Environment impacts the metabolic dependencies of Ras-driven non-small cell lung cancer. *Cell Metabolism* **23** 517–528. (<https://doi.org/10.1016/j.cmet.2016.01.007>)
- Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, *et al.* 2010 Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Molecular Cell* **39** 171–183. (<https://doi.org/10.1016/j.molcel.2010.06.022>)
- Evans T, Rosenthal ET, Youngblom J, Distel D & Hunt T 1983 Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33** 389–396. ([https://doi.org/10.1016/0092-8674\(83\)90420-8](https://doi.org/10.1016/0092-8674(83)90420-8))
- Gonzalez A, Hall MN, Lin SC & Hardie DG 2020 AMPK and TOR: the Yin and Yang of cellular nutrient sensing and growth control. *Cell Metabolism* **31** 472–492. (<https://doi.org/10.1016/j.cmet.2020.01.015>)
- Gottlieb E & Tomlinson IP 2005 Mitochondrial tumour suppressors: a genetic and biochemical update. *Nature Reviews: Cancer* **5** 857–866. (<https://doi.org/10.1038/nrc1737>)
- Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP, Alessi DR & Hardie DG 2003 Complexes between the LKB1 tumor suppressor, Strada/b and MO25a/b are upstream kinases in the AMP-activated protein kinase cascade. *Journal of Biology* **2** 28. (<https://doi.org/10.1186/1475-4924-2-28>)
- Hawley SA, Ross FA, Gowans GJ, Tibarewal P, Leslie NR & Hardie DG 2014 Phosphorylation by Akt within the ST loop of AMPK- $\alpha$ 1 down-regulates its activation in tumour cells. *Biochemical Journal* **459** 275–287. (<https://doi.org/10.1042/BJ20131344>)
- Hunter RW, Hughey CC, Lantier L, Sundelin EI, Pegg M, Zeqiraj E, Sichi F, Jessen N, Wasserman DH & Sakamoto K 2018 Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Nature Medicine* **24** 1395–1406. (<https://doi.org/10.1038/s41591-018-0159-7>)
- Ji H, Ramsey MR, Hayes DN, Fan C, McNamara K, Kozlowski P, Torrice C, Wu MC, Shimamura T, Perera SA, *et al.* 2007 LKB1 modulates lung cancer differentiation and metastasis. *Nature* **448** 807–810. (<https://doi.org/10.1038/nature06030>)
- Leveillard T & Sahel JA 2017 Metabolic and redox signaling in the retina. *Cellular and Molecular Life Sciences* **74** 3649–3665. (<https://doi.org/10.1007/s00018-016-2318-7>)
- Lighton JRB 2008 *Measuring Metabolic Rates – A Manual for Scientists*. Oxford, UK: Oxford University Press.
- Ling NXY, Kaczmarek A, Hoque A, Davie E, Ngoei KRW, Morrison KR, Smiles WJ, Forte GM, Wang T, Lie S, *et al.* 2020 mTORC1 directly inhibits AMPK to promote cell proliferation under nutrient stress. *Nature Metabolism* **2** 41–49. (<https://doi.org/10.1038/s42255-019-0157-1>)
- Maehama T & Dixon JE 1998 The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *Journal of Biological Chemistry* **273** 13375–13378. (<https://doi.org/10.1074/jbc.273.22.13375>)
- Metallo CM, Walther JL & Stephanopoulos G 2009 Evaluation of  $^{13}\text{C}$  isotopic tracers for metabolic flux analysis in mammalian cells. *Journal of Biotechnology* **144** 167–174. (<https://doi.org/10.1016/j.jbiotec.2009.07.010>)
- Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, *et al.* 2011 Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* **481** 380–384. (<https://doi.org/10.1038/nature10602>)
- Mitchell PD 1978 *Nobel Prize Lecture: The Nobel Prize in Chemistry 1978*. Stockholm, Sweden: Nobel Prize Outreach AB. (available at: <https://www.nobelprize.org/prizes/chemistry/1978/summary/>)
- Mukherjee S 2011 *The Emperor of All Maladies – A Biography of Cancer*. USA: Scribner.
- Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS & DeBerardinis RJ 2011 Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* **481** 385–388. (<https://doi.org/10.1038/nature10642>)
- Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, *et al.* 2008 An integrated genomic analysis of human glioblastoma multiforme. *Science* **321** 1807–1812. (<https://doi.org/10.1126/science.1164382>)
- Racker E 1972 Bioenergetics and the problem of tumor growth. *American Scientist* **60** 56–63.
- Racker E 1981 Warburg effect revisited. *Science* **213** 1313. (<https://doi.org/10.1126/science.6765631>)
- Racker E & Spector M 1981 Warburg effect revisited: merger of biochemistry and molecular biology. *Science* **213** 303–307. (<https://doi.org/10.1126/science.6264596>)
- Rous P 1910 A transmissible avian neoplasm – sarcoma of the common fowl. *Journal of Experimental Medicine* **12** 696–705. (<https://doi.org/10.1084/jem.12.5.696>)
- Sagan L 1967 On the origin of mitosing cells. *Journal of Theoretical Biology* **14** 255–274. ([https://doi.org/10.1016/0022-5193\(67\)90079-3](https://doi.org/10.1016/0022-5193(67)90079-3))
- Sanchez-Cespedes M, Parrella P, Esteller M, Nomoto S, Trink B, Engles JM, Westra WH, Herman JG & Sidransky D 2002 Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. *Cancer Research* **62** 3659–3662.
- Spector DH, Varmus HE & Bishop JM 1978 Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates. *PNAS* **75** 4102–4106. (<https://doi.org/10.1073/pnas.75.9.4102>)
- Takeya T & Hanafusa H 1983 Structure and sequence of the cellular gene homologous to the RSV src gene and the mechanism for generating



- the transforming virus. *Cell* **32** 881–890. ([https://doi.org/10.1016/0092-8674\(83\)90073-9](https://doi.org/10.1016/0092-8674(83)90073-9))
- Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D, Leigh I, Gorman P, Lamlum H, Rahman S, *et al.* 2002 Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nature Genetics* **30** 406–410. (<https://doi.org/10.1038/ng849>)
- Vander Heiden MG, Cantley LC & Thompson CB 2009 Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324** 1029–1033. (<https://doi.org/10.1126/science.1160809>)
- Viegas FO & Neuhauss SCF 2021 A metabolic landscape for maintaining retina integrity and function. *Frontiers in Molecular Neuroscience* **14** 656000. (<https://doi.org/10.3389/fnmol.2021.656000>)
- Warburg O 1908 Beobachtungen über die oxydationsprozesse im seeigelei. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **57** 1–16. (<https://doi.org/10.1515/bchm2.1908.57.1-2.1>)
- Warburg O 1923 Versuche an überlebendem carcinom-gewebe (Methoden). *Biochemische Zeitschrift* **142** 317–333.
- Warburg O 1925a Iron, the oxygen-carrier of respiration-ferment. *Science* **61** 575–582. (<https://doi.org/10.1126/science.61.1588.575>)
- Warburg O 1925b The metabolism of carcinoma cells. *Journal of Cancer Research* **9** 148–163. (<https://doi.org/10.1158/jcr.1925.148>)
- Warburg O 1956 On the origin of cancer cells. *Science* **123** 309–314. (<https://doi.org/10.1126/science.123.3191.309>)
- Warburg O & Minami S 1923 Versuche an überlebendem carcinom-gewebe. *Klinische Wochenschrift* **2** 776–777. (<https://doi.org/10.1007/BF01712130>)
- Warburg O, Posener K & Negelein E 1923 Über den Stoffwechsel der karzinomazelle. *Biochemische Zeitschrift* **152** 309–344.
- Warburg O, Posener K & Negelein E 1924 Über den Stoffwechsel der tumoren. *Biochemische Zeitschrift* **152** 319–344.
- Warburg O, Wind F & Negelein E 1927 The Metabolism of tumors in the body. *Journal of General Physiology* **8** 519–530. (<https://doi.org/10.1085/jgp.8.6.519>)
- Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, Schlattner U, Wallimann T, Carlson M & Carling D 2003 LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Current Biology* **13** 2004–2008. (<https://doi.org/10.1016/j.cub.2003.10.031>)

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