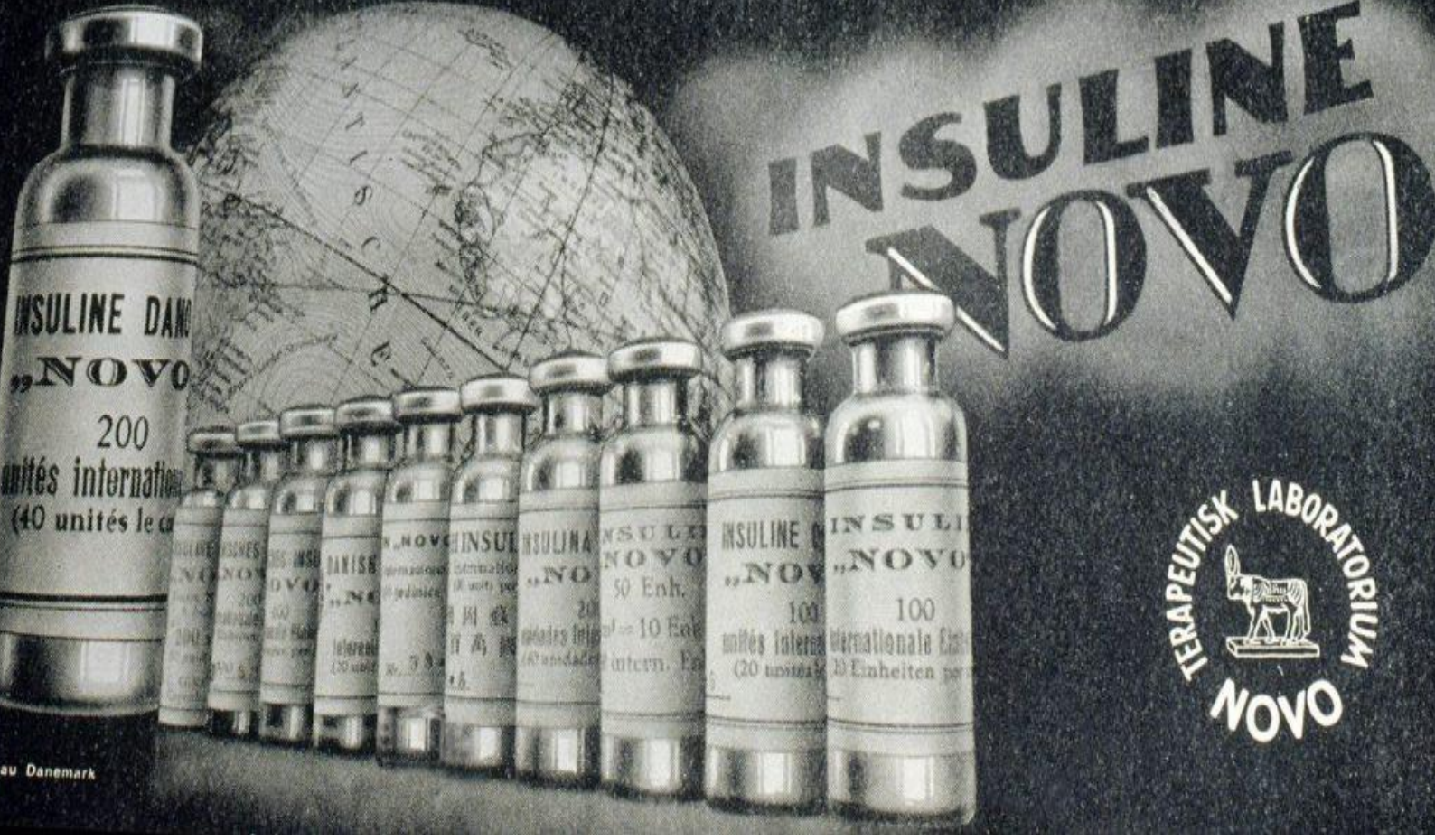

A short history of cell culture media and the use of insulin

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The ancestors of cell culture media were the balanced salt solutions (BSS) devised by early workers interested in studying isolated organs ex vivo.
Such solutions provided irrigation and supply of water, bulk inorganic anions essential for normal cell metabolism, osmotic balance (isotonicity), a carbohydrate such as glucose as energy source, and a buffering system to maintain the medium within the physiological pH range (7.2 – 7.6) usually monitored by the addition of phenol red (1).
The first BSS was composed in 1885 by Sydney Ringer (1836-1910), a British clinician and pharmacologist from University College London who worked on the isolated frog heart (2). A version containing lactate is still used for intravenous perfusion. Ringer's solution was modified by Maurice V. Tyrode (1878-1930), an American physiologist born in Besançon to study isolated kidneys. Another popular balanced salt solution was devised by John H. Hanks (1906-1990), an American microbiologist, in order to attempt to cultivate the leprosy bacillusin vitro (he never succeeded) (3). Renato Dulbecco, an Italian born (1914) American virologist (who won the Nobel Prize in Physiology and Medicine in 1975 with Howard Temin and David Baltimore for their discovery of reverse transcriptase), described the much used Phosphate Buffered Saline (PBS) (4).
However it became clear that in order to get cells to actually proliferate in vitro, additional nutrients had to be added to culture media.
The first successful attempt at tissue culture is attributed to Ross Harrison (1870-1959) at Johns Hopkins University, who explanted in 1907 tadpole tissues in clotted frog lymph as nutritional and growth support, using a hanging drop on a glass slide. He was successful in observing neurite expansions (5,6).

Building on Harrison's success, the major early figure in tissue culture was Alexis Carrel (1873-1944), a French surgeon. He moved to the University of Chicago in 1903 and obtained the Nobel Prize in Physiology and Medicine in 1912 for inventing a method to suture blood vessels. In 1906 he moved to the Rockefeller Institute for Medical Reseach and started his work on tissue culture, in which he did pioneering work (124 articles, 7,8) and dominated the field, for better or for worse, until his death. He adopted Harrison's hanging drop technique but replaced the frog lymph by a plasma clot and fed the explants serum, a salt solution and chick embryo extract, which he called "plasmatic medium", which became a standard until the 1950's. His most famous experiment was the

maintenance for over 34 years of an embryonic chicken heart explant, finally discarded two years after Carrel's death. This nurtured Carrel's firm belief that primary cells in culture were immortal given the appropriate nutrition. Definitive evidence that this is wrong was provided by Leonard Hayflick (born 1928), an American gerontologist and professor of anatomy at UCSF, who demonstrated in 1965 that normal human diploid fibroblasts do not survive in vitro for more than approximately 50 divisions ("Hayflick's limit") (9). The survival of Carrel's culture was likely due to the daily feeding of new embryonic cells from the embryonic extract.

The first continuous culture of a rodent cell line (L929) from a single cell was generated by Wilton R. Earle at the National Cancer Institute in 1943 (10). In 1951, George O. Gey (1899 -1970) at Johns Hopkins University developed the first continuous human cancer cell line from Henrietta Lacks's cervical carcinoma, the celebrated HeLa cell line whose eagerness to grow has resulted in cross-contamination of many cell cultures…
In the early 50s, synthetic media were being developed. By then it had been determined that cell growth required a basal mix of salts, sugars, amino acids and vitamins, plus a supplement of poorly defined biological fluids or extracts such as plasma or serum from various sources (11) which provided a.o. what were later identified as growth factors (see section on insulin-like growth factors (IGFs)), hormones, albumin and transferrin.
Harry Eagle (1905-1992), an American pathologist (Johns Hopkins and NCI) was one of the first to define more precisely the nutritional needs of cells in culture, leading to the media still in use today, such as Eagle's Minimal Essential Medium (MEM) (12) or its modification by Dulbecco (DMEM).

The earliest attempt to get rid of the variability introduced by the need for serum was the chemically defined, synthetic medium (F12) devised by Richard G. Ham at the University of Colorado in 1965 (13).
But the development of serum-free culture methods owes much to the pioneering work of Gordon H. Sato (born 1927), an American cell biologist at UCSD, with David Barnes at the University of Pittsburgh. They developed in the late seventies serum-free media for a variety of cell lines, and showed that the cell lines have different requirements for

hormones, growth factors or other factors such as attachment factors (14). But they found out that there was a rather “universal” growth factor: insulin.

As stated by Barnes and Sato (14), “Although different cell lines have been found to respond to different hormones, and to varying degrees to these hormones,insulin thus far has been found to be stimulatory in serum-free medium for the growth of virtually every cell type examined. In many cases, the concentration of insulin required for good growth is much higher than can be considered physiologically relevant, suggesting that insulin may be mimicking the activity of a related peptide for such cells”.
We know today that indeed the bulk of the growth promoting effect of insulin in cell culture is likely through its low-affinity interaction for the insulin-like growth factor I receptor (IGF-IR) (see section on insulin-like growth factors). However, mammary tumour cell lines like the MCF-7 cell line appear to respond to low levels of insulin, suggesting that in those cells the growth-promoting effect is mediated by the insulin receptor (15, 16).

One advantage for using high concentrations of insulin rather than low concentrations of IGF-I is the lack of binding of insulin to IGF-binding proteins (IGFBPs), which are produced in variable amounts by different types of cells (see section on IGFs) and introduce a factor of variability by interfering with the actions of the IGFs (although there are engineered IGF-I variants with low affinity for the IGFBPs).
Another potential advantage is the conservation of signalling through the insulin receptor and therefore the maintenance of the anabolic effect of insulin, which would not be the case with low IGF-I concentrations. Anabolic metabolism appears to be important for optimal cell growth (17). This benefit may however be transient due to the likely downregulation of the insulin receptor by the high insulin concentrations (18).
For further reading, see ref. 19.

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