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Pre-Banquet Presentation by the Honored Guest on His 75th Birthday

HISTORY OF EFA AND THEIR POSTANOIDS: SOME PERSONAL REMINISCENCES

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To compress this vast subject into 30 min, particularly in the presence of non-technical wives, is a formidable task, but the late Ulf von Euler gave an excellent account of the discovery of prostaglandins at the Golden Jubilee Congress in 1980 and George Burr gave his important history of his early work on EFA in Berkeley and in Minneapolis. Therefore, my task is eased and perhaps I too, on a very much humbler level, may be permitted to reminisce. Since the two classical Burr papers, the literature has become enormous. For instance, Ralph Holman has published 341 papers, about 7×10^5 words. In English measure, they would stretch for 1.2 miles, or in international measure to the length of 500 rolls of toilet paper. Indeed, multiplication of words accompanies the progress (or is it regress?) of civilization as the following table shows:

INFLATION

The Creation (4004 BC)	6 sentences in 144 hours
The Ten Commandments (1491 BC)	10 sentences
The Lord's Prayer (AD 31)	65 words
Declaration of Independence (1776)	1,332 words
US–Soviet communiqué on agreeing not to reach agreement on arms agreement (1984)	2,734 words
EEC Directive on existence of the butter mountain (1985)	5,317 words
Crawford on the developing brain needing EFA (9 papers)	20,132 words
Holman on *trans* EFA being nasty (16 papers)	71,738 words

I shall start at the beginning and pay little attention to the last 30 yr, so all who hope for mention of their important work can now sleep. About 3,500 yr ago, the Israelites were bored by making bricks without straw, and Moses (or perhaps Aaron) hit the sea with a rod—a poor tool for that purpose, but the Lord was on his side. All the fish died. Shortly thereafter, Pharoah had an infarct followed by an epidemic of hardening of the heart amongst the Egyptians. This is the earliest indication of the importance of fish in preventing myocardial infarction. We leave the Old Testament, noticing in passing that if a piece of uterus had happened to be lying around when Onan, son of Judah, spilt his seed upon the ground, the observation of Kurzrok and Lieb in 1931 might have been anticipated by 2.5 thousand years, and all those squiggles of prostanoids we have seen on slides would have been inscribed on rock.

There have been several unethical experiments on the production of deficiency of EFA in helpless infants—von Groer in 1919, Holt in 1935, von Groer's nephew, von Chwalibogowski, in 1937. So I mention with relief the first ethical production of deficiency. St. Jerome tells us that St. Hilarion (who lived about 200 AD) from the 31st to 35th year of his life lived daily on 6 oz of barley bread and vegetables slightly cooked without oil; but finding that his eyes became dim ($n - 3$ deficiency) and his body shrivelled with a scabby

eruption and dry mange ($n-6$ deficiency), he added oil to his diet and lived healthily to his 80th year.

The need for fat in the diet was complicated by unsuspected deficiency of fat-soluble vitamins, by the demonstration in 1847 by Lawes and Gilbert that carbohydrate gave rise to fat, and by the difficulty of removing EFA. Aron in 1919, Osborne and Mendel in 1920, Drummond and Coward in 1921 tried unsuccessfully to demonstrate essentiality. I turn to Berkeley in 1924 where H. M. Evans had been professor for 9 yr. I believe he and Warburg were the two greatest biochemists of this century (Chauncey Leake wrote that Evans should have received four Nobel Prizes and George Corner agreed). In 1924, upon receiving his Ph.D., George Burr joined Evans who had recently made the first active extract from the pituitary (growth hormone with Joseph Long in 1921), discovered vitamin E (with Scott Bishop in 1922), and was doing classical work on the oestrus cycle in rats. In 1927 Evans and Burr discovered "A new dietary deficiency with highly purified diets", using sucrose in place of highly purified starch which had been shown to contain some non-extractable lipid. In the same year, a second paper suggested that there was "an unknown member (F or H) of the vitamine class'. The next year a third paper showed "The favorable substance in fats—possibly a new vitamine (F) . . . can be recognized in the fatty acid portion after saponification". George Burr married one of Evans' technicians, Mildred Lawson, moved to the Department of Botany in Minneapolis, and with her published the two classical papers in 1929 and 1930. They thought arachidonic acid was inactive, but Osmo Turpeinen (whose wife Kaisa was working with Evans on the oestrus cycle) showed in 1937 in Evans' department that it was even more active than linoleic acid.

It was in this year, 1937, that I went to the U.S. to see the work being done on EFA. As a student, I had noticed what I would still regard as the most challenging fact in clinical medicine: the expectation of life of a middle-aged man in the U.K. had hardly changed since William Farr first estimated it in about 1841, and the same is true of the U.S. since the beginning of this century. But the diseases killing middle-aged men have changed dramatically: then they were pneumonia and tuberculosis; the Western diseases were rare as they are today in the Third World though increasing. I thought the rise must be nutritional, probably mainly caused by changing the unstable EFA to stable saturated fats as we fed animals in unnatural ways and increasingly processed and sophisticated foods. It was on this that I wanted to work. So, I refused offers of jobs to be taken up when I qualified, from Sherrington in 1932 and Florey in 1935. The latter told me I was a fool, as human nutrition was uninteresting, and he appointed Chain. When Florey had cleared up antibiotics and turned to atherosclerosis, on which he gave his Presidential Address to the Royal Society, he suggested we should spend a couple of hours together over lunch and opened the conversation by saying he wanted to apologize because he had been wrong, and he now found human nutrition interesting. His biographer told me I am the only person to whom Florey ever apologized. So, in the unlikely event of my being allowed the luxury of a tombstone, engraved on it will be: "Here are the remains of the chap to whom Lord Florey apologized".

While a student in 1934, I had encounters with a third Nobel Prize-winner and a fourth who thought he should have been, namely Sir Edward Mellanby, who in that year published a book, *Nutrition and Disease*, in which he attributed a wide variety of totally distinct diseases of the nervous system to deficiency of vitamin A. In an anonymous review in a student magazine, I wrote that it was the worst book on nutrition ever written by an intelligent man. Mellanby, recently head of the Medical Research Council, did not agree, and discovering the authorship, summoned me. I fought back. I pointed out that multiple sclerosis (in the epidemiology of which I had become interested through friendship with Sir Francis Walshe) was virtually unknown in India and China, countries in which deficiency of vitamin A was common. It is interesting that, in his supposed successful therapy in this disease, Mellanby had used vitamin A dissolved in cod-liver oil; we are at present taking part in a trial of long-chain fish fatty acids in this disease which in 1956 I suggested was related to EFA, after my friend Roy Swank had in 1950 suggested it was caused by high total fat.

My other encounter in 1934 was with Dr. George Minot whom I had met in November in London, a year after he had published in Cowdry's *Atherosclerosis* a 12-page chapter with Soma Weiss on nutrition and atherosclerosis, hardly mentioning fat but discussing, for instance, the work of Thomas (1927) on the absence of cardiovascular disorders in Eskimos. In the same book, Anitschkov had a masterly chapter of 46 pages describing his own work—he had first in 1911 produced atheroma in rabbits by feeding cholesterol. Dr. Minot told me that if I thought dietary fat was important in atherosclerosis and coronary heart disease I should come to work with him in Boston when I qualified, and I accepted his formal invitation for a year. The following year, 1935, I visited Anitschkov in Leningrad and met him again in 1938 when, with the pathologist, Dr. Robb-Smith, I visited Aschoff who in 1906 had supported Virchow's inbibition theory and persuaded Windaus to demonstrate for the first time in 1910 the presence of cholesterol in atheroma. Anitschkov was then working with Aschoff.

When I was a student, University College in Oxford had offered me a fellowship to be taken up on qualification, which required that I teach its students during my studies, and which allowed me a year to travel. When I qualified in 1937 and was given a travelling scholarship, I migrated to Magdalen College at Sherrington's suggestion as Fellow in succession to Sir John Eccles, and Magdalen wanted me in residence as soon as the long vacation of 3 months was over. So after spending only a few days with Minot, I went to San Francisco to see the work of Evans, McQuarrie, Lepkovsky, Hansen and others. I became a close friend of Evans, and the following year (1938) he, John Fulton of Yale and I stayed on the Lake of Geneva with Dr. Arnold Klebs in his château. The only work on the subject in the U.K. at this time was that so excellently done by Ida Smedley-Maclean and her colleagues at the Lister Institute whom I used to visit; she published her first paper (on the synthesis of fats) the year after I was born.

During the 1939–45 War, in which I carried out nutritional surveys for the Ministry of Health, we did some estimations of EFA with thiocyanogen numbers and iodine values, and I had the opportunity in 1944 when carrying out some work for the Royal Canadian Air Force to visit Canadian Indians and Eskimos, taking a slit-lamp microscope so that with doubly polarized light I could detect very early arcus senilis. Even old natives had no trace, whereas 10% of young British pilots being trained at the same latitude as the Indians had it. Apart from the work of Thomas I have mentioned, Rosenfeld had shown in 1902 that the fat of a 12-yr-old Eskimo girl had an iodine value of 79 as compared with 63 for German children of the same age, and Corcoran and Rabinowitch had shown in 1936 that Eskimos had low plasma cholesterol. Snapper in 1941 (second edition, 1965) discussed the difference in atherosclerosis between Chinese and Westerners, remarking that "The Chinese diet contains only small amounts of cholesterol but considerable quantities of unsaturated acids, especially of linoleic and linolenic acid. It is certain that the average cholesterol content of the blood of the Chinese is lower than that of Westerners and this gives perhaps an indication why the tendency to lipoid infiltration of the vessel wall is so much smaller in the Chinese".

At the end of the War, I had teams in Holland and then in Germany. I had in my University Laboratory of Human Nutrition the brilliant Indian, Professor Ramalingaswami, as a postgraduate student working on the pathology of EFA deficiency, particularly the skin; but we showed changes in the sebaceous glands that might be relevant to acne, and in cartilage that might be interesting in relation to connective tissue disorders and osteoporosis; and we found sludging of the blood. He was followed by Professor Basnayake from Sri Lanka, who did excellent work on the deposition of cholesterol in deficiency of EFA. In pure deficiency (i.e. a fat-free diet), cholesteryl oleate accumulated in the avascular epidermis despite the plasma cholesterol being lower than in pair-fed control rats, but in a relative deficiency, i.e. a low ratio of EFA to certain non-EFA (long-chain saturated and *trans* isomers) as in Western diets, plasma cholesterol was abnormally high and the accumulation in epidermis was enhanced (we used epidermis as being analogous in this context to intima of which in the early 1950s there was insufficient for the analytical methods then available). At that time, 1951, Ancel Keys came to work

in my Department for 2 yr, but that was when he considered all fats equally harmful—that corn oil raised plasma cholesterol like any other fat. Tuttle in 1950, after 15 yr of study of 280 hypercholesterolaemic patients (mainly thyrotoxic), concluded that there was "A new lipotropic agent . . . selective in lowering blood cholesterol. It is an unsaturated fatty acid split away from lecithin derived from sunflower seed oil (Povon 9,12-octadecadienoic acid)". Two years later, 1952, Larry Kinsell and Jay Groen with their colleagues independently showed that saturated fats such as butter raised plasma cholesterol whereas vegetable oils lowered it; Groen's paper was given in Amsterdam in the presence of Ancel Keys who said it was wrong, and of me who supported it from our work on rats. Careful work by Ahrens confirmed Kinsell and Groen but led to controversy with me (and Kinsell) in 1956. I maintained (and still maintain) that the only dietary fatty acids that tend to lower plasma cholesterol are the EFA of the 2 families, but because the fatty acids of menhaden oil lowered plasma cholesterol but did not cure the skin lesions of EFA-deficient rats, Ahrens (and Thomasson) maintained these fatty acids were not EFA and the lowering was proportional to the iodine values. This is obviously wrong, and it is established that elaeostearic acid (a conjugated isomer of linolenic) raises plasma cholesterol in the rat and in man.

But atherosclerosis and ischaemic heart disease (IHD) are not synonymous: in some countries (Jamaica and Cuba where coconut oil rich in the atherogenic but non-thrombotic medium-chain fatty acids is eaten), the former is common but the latter is rare. The decline in deaths attributed to IHD in European countries in the 1939–45 War would be likely to be a decrease in thrombosis rather than an alteration in the chronic process of atherosclerosis. In 1941 Macfarlane and colleagues in Oxford showed that the clotting time of plasma in the presence of Russell's viper venom was determined partly by some substance present in chylomicra and crude lecithin. He gave me some of the latter which was very active in curing EFA-deficiency in rats. My friend and former pupil, John Poole, who worked with Macfarlane, showed in 1956 that phosphatidylethanolamine was almost certainly essential for blood coagulation and that it was very active if both fatty acids were saturated. In that year, I suggested that as phospholipids were known to be increased in the serum of persons with IHD, perhaps unusually saturated fatty acids might make such compounds persist for a longer time and so increase the thrombotic tendency of blood. Bill Connor worked with John Poole in Oxford and together they published an important paper in 1961 showing that the thrombotic tendency of blood (using Chandler's tube) was increased by palmitic and particularly by stearic acids, but decreased by linoleic and arachidonic acids. The last compound had a sentimental interest for me since in 1954—5 yr before Osbond's synthesis—Ernst Klenk isolated pure arachidonic acid, sealed it under nitrogen and brought it to Oxford for me to use in some "important experiment"—so hopeless a request that I kept it refrigerated until Poole asked for it. A little later Renaud (1965) and Nordøy (1968) began the splendid work they have done in the past two decades. At the same time (1962) Professor Notevarp in Trondheim and I were in contact because of our interest in timnodonic ($20{:}5n-3$) and clupanodonic ($22{:}6n-3$) acids, and at the beginning of 1963 I began contact with British Cod Liver Oils. In my enthusiasm for these, I started to breakfast on cod livers canned in cod-liver oil, on toast, and persuaded my friend, Alex Poniatoff of Ampex, to do the same. I also encouraged him to finance Larry Kinsell's work as well as Deuel Conferences. We both found one can was too much to eat, and that by the following day peroxidation had occurred despite refrigeration. Notevarp believed that linolenic acid could not be desaturated and elongated by man, though I disagreed since Klenk and Bongard (1952) had shown the large amounts of clupanodonic acid in brain, and in pure vegetarians there would be no dietary source of this. From their work, I had always considered the linolenic family ($n-3$) to be a second class of EFA. Ralph Holman had proved that linolenic acid could give rise to those long-chain fatty acids, just as Jim Mead and his colleagues had in 1956 shown the conversion of linoleic to arachidonic. These conversions were discussed in detail by Holman, Klenk and Mead (order is alphabetical) at the first international conference on EFA, which I organized in Oxford in 1957 as the 4th ICBL. And here I want to say how

much we owe our knowledge of the chemistry and metabolism of EFA to those three distinguished workers, as well as to Unilever Research in Vlaardingen previously led by Jan Boldingh and David Van Dorp.

At the time that Professor Notevarp and I were exchanging views about 20:5 and 22:6$n-3$, we started to do some human tests of $n-3$ EFA in platelet adhesiveness, using the Salzman glass-bead technique. Wayne Martin, a U.S. chemical engineer, had visited me in Oxford in 1959 and often for the next 7 years. With the aid of the Minnnesota Linseed Oil Company, an oil containing 60% linseed oil and 40% soybean oil was produced which lowered platelet adhesiveness, whereas the flaxseed oil Owren was then using did not. Dr. Clement Martin and Dr. Kirby carried out a number of tests in the New Jersey State Hospital, and we realized the enormous importance of preventing peroxidation of these highly reactive fatty acids. This was alarmingly demonstrated on me when, after having the privilege of working with Drs Bang and Dyerberg on Eskimos on NW Greenland and being unable to do bleeding times because our visit was curtailed by weather, I went on an Eskimo diet myself for 100 days, eating considerable amounts of seal blubber and undeodorized mackerel oil. Despite a daily supplement of tocopherol, my malondialdehyde (as estimated by TBA) rose to about 150 nmol per ml serum (normal, 3) and 50 nmol daily in urine. MDA is teratogenic, but because the diet also caused disappearance of spermatozoa I will not have mis-shapen offspring.

The field of the 5 classes of prostanoids is a very intriguing one: apparently they can come from 20:3$n-6$, 20:4$n-6$, 22:4$n-6$, 20:5$n-3$ and 22:6$n-3$, though only very slowly from the docosaenoic acids. To prostaglandins, thromboxanes, prostacyclins and leukotrienes, we now must add lipoxins and hepoxilins, and the recent triplicated Nobel Prizes rightly emphasize these brilliant advances. The control of the production of the different compounds by one another is a fascinating subject on which Rodolfo Brenner, Bill Lands and others are doing such excellent work. I doubt if there is much future in *Oenothera* as a source of γ-linolenic acid since *Spirulina* grows much more easily—on sewage, which with the emphasis on dietary fibre is not in short supply. So we need much more research as I recall was emphasized years ago in a speech in *King Henry V*, Act III that is not irrelevant to this Second Congress superbly organized by Michael Crawford.

CONGRESS II

SCENE I. Hatfield House. Before the banquet.

Alarms. Enter Ralph Holman, Vane, Crawford, Samuelsson, Lands, Lewis, Schettler, and many others, with wives and camp-followers.

R. Hol. Once more unto the labs, dear friends, once more;
Or heart disease will make your English dead!
In health, there's nothing so becomes a man
As low cholesterol, high EFA:
But when non-EFA flow in our veins,
They imitate the action of snake-venom,
Stiffen the vessels, thrombose up the blood,
Joining blood platelets with hard calcium links:
This lends the wall a terrible aspect;
Lets plaques push through endothelium
Like the grass mole-hills; lets cholesterol swell it
As fearfully as does a fatty streak
O'erhang and infiltrate the intima,
Swill'd with the wild and turbulent blood-stream.
Let prostacyclins keep the platelets wide;
Hold hard thromboxanes, drink a little spirit
Against angina!—Oh, infarcted English,

Whose blood is full of saturated fats,
Cholesterol whose esters with wrong lipids
Have in these parts accumulated thus
Insoluble for lack of double bonds.
Dishonour then your parents who did give
You hyperlipoproteinaemic genes!
Don't copy now the men of grosser blood,
But teach them how to eat. And I, Ralph Holman,
With home in Minnesota, shew you here
The value of my research; let us swear
That you'll reform your feeding, which I doubt not.
For there is none of you so lean and sparse
That hath not senile arcus in your eyes.
I see you seated, hungry, bored, asleep,
Draining an empty glass. Dinner's afoot.
Refill your glasses and let each one say
"Thank God for prostanoids and EFA!"