COMMISSION OF THE EUROPEAN COMMUNITIES

SYMPOSIUM

on

NEW VARIETIES OF COLZA

BRUXELLES - 11-12 April 1978

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Printed in Belgium

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Symposium on New Varieties of Colza

Organized by the Commission of the European Communities
Brussels - 11-12 April 1978

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SYMPOSIUM ON NEW VARIETIES OF COLZA
11 April 1978

Speech by Mr. Waechter - Chairman

On behalf of the Commission of the European Communities, I welcome you all to Brussels and hope that the objective of this symposium will be achieved.

Allow me to recall briefly the Commission's reasons for organizing this symposium.

Since you are all more or less closely involved with the product which is our principal topic you will be aware that colza oil consumption has fallen in the Community and has not yet regained its normal level. It is obvious that this drop in consumption is not due to economic reasons; as for instance the price level, or to a fall in oil consumption in general, but rather to the publication of studies showing that the erucic acid content of colza oil had contributed to health troubles in experimental animals and that, consequently, the possibility of such troubles also arising in human beings could not be ruled out.

The farmers reacted by adapting their crops, on a scale and with a rapidity which in my opinion has not been rivalled in any other product. New varieties were produced, not without risk for profits. Today, Community colza production for food purposes is all of varieties with a low erucic acid content.

This symposium, therefore, is an attempt to give replies to questions concerning the qualities of the oils obtained from these new varieties.

Today we do not want to determine whether the charges against the old varieties were justified or not, we merely want to investigate the situation as regards the new varieties.

I wish to emphasize the fact that the Commission, in organizing the meetings, is approaching the matter with an open mind, since it must keep a strictly neutral stance. Its hope is that the symposium can make the results of research work on the new varieties available to consumers.

For this reason, this symposium is meant primarily for consumers both inside and outside the Community who purchase Community colza oil. We thought it advisable to organize the symposium at this time in order to avoid incomplete discussion or argument arising again before the problem as a whole had been studied in the light of scientific and medical experiments.

For this reason we have invited independent scientists to inform us of the results of experiments on the colza oil obtained from the new varieties, which will allow us to draw clear conclusions accessible to all.

This is not a scientific gathering, but rather an attempt at communication between scientists of various disciplines and consumers.

We realise that it will not be easy to achieve this objective and we cannot ignore the fact that difficulties of comprehension are likely to arise. For this reason we have specifically requested the scientists, whenever possible, to use simple language which can be understood by non-specialists. With these few words I have tried to outline our objective.

Now I have pleasure in introducing Mr. André François, who will be responsible for conducting the symposium.

In his capacity as Director of the National Centre for the Coordination of Study and Research on Nutrition and Food, Research Director at the National Institute of Agricultural Research (where he set up the Nutrition Department), member of the Higher Council for Public Health in France and Advisor to the General Delegation on Scientific and Technical Research, Mr. André François has been in constant touch with all the work carried out on colza, at the stages of both seed and oil production, in particular work on the new varieties.

I have the honour of citing the names of the European organizations which have been invited :
European Bureau of Consumers' Union (BEUC) - Committee of Family Organizations in the European Communities (COFACE) - European Trade Union Confederation (CES) - European Community of Consumers' Cooperatives (EUR CCOP) - Committee of Agricultural Organizations in the EEC (COPA) - General Committee for Agricultural Cooperation in the EEC (COGECA) - Seed Committee of the Common Market (COSEMCO) - Association of the Margarine Industry of the EEC countries (IMACE) - Federation of the Seed Crushers and Oil

Processors in the EEC (FEDIOL) - Association for the Oilseeds, Animal and Vegetable Oils and Fats and Derivatives Trade in the EEC (ANGO) - European Chain-Stores Association (GEMAS).

I would also point out that to his great regret, Mr. Gounelle De Pontanel, who was to represent the Scientific Committee for Foodstuffs has been kept in Paris by a prior commitment.

SYMPOSIUM ON "NEW VARIETIES OF COLZA"

Opening address by Mr André François

Director of the Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation (C.N.E.R.N.A.) (National Centre for the Coordination of Study and Research on Nutrition and Food)

Rapporteur

12 April 1978

Mr. Chairman, Ladies and Gentlemen,

Firstly, I should like to say that I feel very honoured to have been chosen as Rapporteur of this important information meeting; I should also like to thank the Chairman for his kind words.

If I have accepted the Commission's invitation it is because I was one of the first nutritionists to become interested in colza oil, mainly as a result of research carried out in the sixties by the Finn, Roine. This researcher showed that a number of oils, among them colza and soya, affected the heart of experimental animals.

In France these results attracted the attention of both the Cooperative Laboratory and the National Institute for Agricultural Research (INRA). Thus the first studies by Jean Causeret and Gérard Rocquelin were carried out at my request when I was head of the Nutrition Department of INRA. It has been a long and difficult task to arrive at the situation we have today, where we know the facts about colza oil.

Extending a little our subject area, I should like to say that in many respects the case of the colza plant is an exemplary one and a type of experimental model illustrating perfectly what nutritional policy should - and must - be.

The underlying objective of nutritional policy is to ensure a satisfactory balance between food production and the consumption of food products, whereby priority should be given to the protection of health; clearly in this respect the concern of health specialists and nutritionists coincides perfectly with that of consumers.

To this end, the primary objective is to ensure that each individual receives the necessary minimum of food, which implies the production of foodstuffs in sufficient quantity which are accessible to all from the economic point of view. At the other extreme, it also means avoiding the food surpluses which are frequent in our industrialized societies. In short, one of the objectives of food policy is a quantitative one.

The second objective is qualitative : care must be taken to ensure that the products released to the market have no undesirable effect on consumers' health and it is this aspect which we will deal with today with reference to colza oil.

However, food policy must be geared to the long term. To govern, it has been said, is to anticipate, but in the case which concerns us the question is to anticipate what ?

The question of food resources on our planet is a preoccupying one, since the world population is increasing very rapidly. Some figures are in order here : at the beginning of our era, two thousand years ago, the population of our planet was between 100 and 200 million. In 1830 this figure was 1 000 million. In 1930, a hundred years later, the world population was 2 000 million and at present it is almost 5 000 million and growing daily. The world food situation is a cause for concern, with an imbalance between the various regions of the globe. In some, wheat and vegetable protein are given to animals, whereas in others there is not sufficient of these foodstuffs for man. Added to this is the fact that animals are bad processors of energy and protein.

For all these reasons it is wise not to ignore the situation and to seek solutions. In the shorter term there is the economic problem of Europe's protein supply.

Now, for nutritionists, oil-bearing plants are particularly interesting since they supply energy through the oil which they contain and indispensable proteins through the oil cakes, which are the residues left after oil extraction.

The case of colza in particular is very interesting. Colza is a model from the point of view of the quality of the proteins which it contains, since their constitutive amino acids exist in substantial and balanced quantity.

In the long term, therefore, two types of use can be envisaged for colza : on the one hand its current use as animal feed and, on the other, its use as a source of protein in human food.

The technologies already available for other oil-bearing products could be applied to colza.

Consequently nutritionists are very interested but, as you know, they are very exacting and therefore they have imposed strict constraints.

Indeed, colza contains anti-nutritional substances which must be eliminated. This is already done in the case of soya, which also contains undesirable substances. In the case of colza there are goitrogenic substances which affect the thyroid gland of animals and man.

There is a solution to this difficulty; in the course of these two days we shall see that man is now able to produce colza varieties free from undesirable substances. We consider the case of colza an exemplary one, since it bears witness to a reasearch effort designed to improve a plant which may constitute an important component of that policy which we can call the food security policy, the aim of which is to ensure the requisite minimum of food for all mankind.

The case of colza is exemplary also for another reason : to my knowledge it is the only product for which there is a tradition of consumption, and where its use as a human foodstuff has nonetheless been called into question. Colza oil has been used in many regions of the globe for a long time : in my own country, but also in India, in China, in Eastern Europe and in Scandinavia. Of course rules have also been applied to other products of common consumption. Without dwelling on the case of alcohol, we know for example that it is not desirable to consume too much calves' sweetbread, which contains substances not to be recommended for those suffering from rheumatism and that liquorice contains an active principle which can raise arterial blood pressure.

However, the sale of these products has not been called into question. In the case of colza oil it was decided to draw up a new file requiring more than ten years' research effort. All this research required a very substantial budget contribution from the various laboratories around the world interested in the problem.

The results which will be outlined in the course of these two days bear witness to this enormous research effort designed to improve a product.

There are still further reasons why the colza case is exemplary; amongst other things, it has enabled researchers to progress in their methodology and to reflect, for example, on the difficulties of transporting results obtained with animals to the defence of human health. Thus, it has been shown that the physiopathological reactions of the various categories of animals to colza oil may vary greatly according to whether the study is of the rat, the pig or the monkey. Even within one species, that of the rat, there are differences between strains. Similarly, the method used for the histopathological examination of organs has been improved.

Of course, where research is concerned, nothing is ever finished. A time does come, however, when the accumulated knowledge is sufficient to make it legitimate to sum up and to draw practical conclusions.

It would appear that this moment has arrived in the case of colza oil. As you pointed out, Mr. Chairman, this is an information meeting and I am sure that the speakers, who are researchers, will make an effort to adapt their language to the audience, which, as you also pointed out, is not an audience of specialized researchers using a common specialized vocabulary.

To make sure that the information is very clear, the talks will be followed by discussions. I should like to stress the fact that participants should not hesitate to ask the speakers lots of questions, so that nothing remains unclear and the position with regard to colza is perfectly understood. Thank you in advance for your cooperation.

Thank you, Mr. Chairman

SYMPOSIUM ON NEW VARIETIES OF COLZA

Address by Mr. G. Schiratti

Head of Division, responsible for the organization of the market in oils and fats

Mr. Chairman,
Ladies and Gentlemen,

I must admit I am a little apprehensive about addressing you today: for one thing it is never easy to speak first, especially when one knows what eminent speakers are to follow, and for another the subject of my address is somewhat uninspiring - figures never raise much enthusiasm from an audience. To illustrate what I am going to say I have circulated four tables, which I would like you to look at as I refer to them.

Before beginning on the figures, I though I would briefly trace the history of colza. My own investigations and the sources I have consulted have led me to conclude that while Mediterranean civilization, especially that of the Greeks and Romans, burgeoned under the sign of the olive, Chinese civilization was nurtured - albeit to a lesser extent - on colza. A number of authors hold this view. Although some of the theories go too far, there seems to be no question that for many centuries China was the world's leading producer of colza seed. If you look at the tables, you will see that in 1934 and 1938 China was still the world's principal producer, supplying two and a half million tonnes of the world's output of about four million tonnes. More recently, although its output has dropped in both absolute and relative terms, China is still a leading colza producer.

However, the theory that in ancient times colza spread first in the Far East has been disputed by a number of European authors. For instance, there is a nineteenth-century French author called Alphonse de Candolle - and we all know how objective French authors are - who claimed that colza originated in Europe, more specifically in Scandinavia, and in Siberia. From Siberia, it then spread gradually through Southern Europe and to certain Asian countries, Mesopotamia, the Middle East and only then to the Far East and China.

De Candolle based his conclusions on a very interesting philological study in which he sought out the word for colza in the ancient languages spoken by the various tribes or ethnic groups: he found that the word occured most frequently in the ancient European languages, in Hebrew and later in Arabic; he found no terms for colza in old Chinese. None the less, even de Quandol admits that colza had spread through China and Japan somewhat before the Greco-Roman period.

Turning from this brief historical and philological digression to the continent of special interest to us - Europe - we find that colza production did not begin to really develop until the eighteenth century, although it had been known since neolithic times. Eighteenth century French and Belgian sources show colza production to have been considerable.

Official statistics have been available in France since 1840; at that time the areas under colza were estimated at 174 000 hectares, with an output of 160 000 tonnes. Some years later, in 1878, statistics indicate that 190 000 hectares were devoted to colza production in Germany.

The plant was cultivated partly for food, especially in rural areas, and partly for other uses, above all lighting. With the advent of gas lighting, and then electricity, colza production in Europe gradually declined until the outbreak of the first world war, during which it again increased, only to drop back to its lowest levels once the war was over; the main reason for this was the abundance of oilseeds from Africa, particularly groundnuts. By 1933, only 5 000 hectares were being cultivated in Germany and in 1939 the figure was the same for France.

During the second world war, colza production once more expanded considerably. I have consulted statistics for that period, but prefer not to quote any figures because I think they speak only half the truth. As you know, farmers at that time tended to declare much less than their real output in order not to get involved in voluntary or compulsory storage, on government instructions, for supplying urban populations. Consequently, while the official statistics for this period do show a steep increase, it may be assumed that the real increase was at least twice the official figure, or even more. Why such a sharp upturn in the second world war ? Because in Europe, torn apart by a murderous conflict, discovered the importance of oilseeds to supplement butter and olive oil supplies and help to meet the population's demand for oils and fats.

Although we all dearly hope that the 1938-1945 situation will never recur, it is not unreasonable to consider carefully what consequences a similar situation might have for the European economy and the food supplies of Europe's consumers - if, that is, world supplies were cut off and we had to rely on indigenous raw materials. The war years have, naturally enough, been forgotten in many countries: they were so tragic, so traumatic for all who lived through them - including myself - that we have tried to forget them and to act as if they would never happen again.

Unfortunately, history shows that mankind never learns from experience and that past mistakes offer no guarantee that new ones will be avoided.

At the end of the second world war, some member countries, such as Germany and France, considered that colza production ought to be supported. But very different methods were used. France had to solve the problem of imports of groundnut oil from its colonies, so it suported colza by isolating the market from that of oil from seeds other than groundnuts. In Germany, where economic policy was more liberal and where there was not the same need to conclude preferential agreements or contracts with particular developing countries, the method chosen was the compulsory incorporation of a certain amount of colza oil in margarine.

As you know, the European Economic Community was set up in 1958 and the market organization in the oilseeds sector was only adopted nine years later. Rome was not built in a day and, understandably, the same goes for our common agricultural policy; I might even say, along with the Italians, that the common agricultural policy is rather like St. Peter's in Rome, begun several centuries ago and not completed because there is always something new to add, something to improve or something to do again. Our position is indeed rather similar: we have been building our edifice bit by bit, we have not finished it yet and we shall certainly not finish it in the immediate future.

Once the problems of the common organization of the market in the main products such as cereals, milk and sugar were settled, or at least defined, and we came, in 1964-65, to consider the policy that should be adopted on oils and fats, two problems faced us; whether we ought to establish a common organization of the market in oilseeds, particularly colza, and if so, what form of common organization would be the best.

The Community decided that colza production should be supported, for reasons that I will try to summarize briefly. Firstly, as colza is an excellent rotation starter crop, especially in some areas of the Community, in agronomic terms we were doing a disservice to agriculture by encouraging or trying to encourage the cessation of colza production. Secondly, at the time colza production was, for economic reasons, increasing and the demand for oil and colza cake was rising even faster. This demand had to be met and as this could be done from Community production, there was no reason to cease colza cultivation and oblige our manufacturers and consumers to meet their needs with imported goods. A third reason was political, namely that it was necessary or desirable to continue national policies, especially as the objectives appeared to be justified and rational.

The support system that we adopted was completely different from that applied by some Member States, notably France and Germany.

In view of the interdependence of the markets for the various oilseeds and their oils, the Community's low degree of self-supply in oilseeds, the need to maintain fair prices for consumers and, lastly, certain international commitments towards EEC-associated countries and under GATT, it was decided that the best system would be to ensure free importation of all oilseeds, including colza seed, together with unlimited importation, subject to moderate customs duty, of all oils for human consumption, including colza oil. In our opinion, this system offers many advantages. I will mention only those of relevance to consumers, because they are our main subject of concern today. I think our policy benefits consumers for two reasons. First, they can buy the end product - oil - at a price which is not linked to European production costs because the direct aid paid to producers offsets the difference between their cost price and the going world rate for oil. There is a very real difference between asking the consumer to pay a price which is fair to the producer, and having that price paid from a Community or national budget. Secondly, our system allows unlimited imports of seeds and oils and our production aid offsets the difference between the price due to the producer and the price at which colza seed or oil could be sold on the Community market against competition from rival oils, the consumer can choose the product which seems to him the most advantageous: the common agricultural policy does not force his hand, does not influence his choice.

Eleven years have gone by since the common organization of the market in colza was set up, and I think the results are broadly satisfactory. Our policy has been accompanied by an increase in Community production, which has benefited our farmers, and an increase in consumption, which means that it has taken effective account of consumer demand and ensured that such demand is met without great difficulty.

In the tables I have circulated you will find confirmation of what I have just said. Colza production (Table 3), which was 579 000 tonnes at the beginning of our market organization in the Community of six, amounted to 1 million tonnes in 1974, while total colza production in the Community of Nine in that same year amounted to about 1 200 million tonnes.

Looking at the pattern of colza consumption in the same period (Table 4), we find that it rose from 267 000 tonnes in 1967-68 to about 490 000 tonnes in 1971-72, after which it began to drop as I shall explain in a moment.

I would like to point out that colza gives two products: oil and cake. For cake, there is no problem. The sale of all the cake produced in the Community has never raised any difficulties and, in our opinion, is unlikely to do so in the near future. In 1973, we saw the beginning of the erucic acid cricis. It started in Italy, and then the campaign against colza oil, on the grounds that the erucic acid it contained was harmful, spread to other countries and more particularly France. I am in no position to say whether this campaign was justified: I will simply say that the economic effect was an immediate and spectacular fall in colza oil consumption (cake consumption naturally remained unaffected).

In Table 4 you will see that colza oil consumption, which had been at a peak of about 490 000 tonnes in the Community of Six in 1971-72, dropped to 276 000 tonnes during the 1975-76 marketing year in the Community of Nine. So the fall in consumption was exceptionally sharp.

Production problems then arose because, although there was some time lag, output necessarily declined. Problems were also encountered, as we shall see, in the marketing of the oil. In these circumstances, Community producers decided to tray and switch from varieties with a high erucic acid content to varieties with a low content. This required a considerable technological effort, but perhaps above all a psychological effort:

you all know how difficult it is for farmers to switch from a known and predictable crop to one that presents agronomic and technological problems. That such an attitude is justifiable is best illustrated by the fact that, in the early days, the new varieties of colza with their low erucic acid conten content were less productive and less hardy. I am no specialist in genetics, but I have the impression that most of these problems have now been solved. However, production has not yet picked up again and recovery is proving difficult.

On the other hand, consumption is showing promising signs of recovery. Looking at 1975-76 (Table No 4) compared with 1976-77, we find that consumption in the Community of Nine has risen from the all-time low of 276 000 tonnes to the more reasonable level of more than 400 000 tonnes. And we hope that once consumers have realized that the new varieties of colza are becoming more widespread and their fears concerning the high erucic acid content have been completely dissipated, consumption will continue climbing even further.

Amidst all these difficulties, there is one factor that seems auspicious and reassuring. Whereas colza oil consumption dropped in the Community, our exports to non-member countries increased despite the fact that they were not subsidized. Looking at Table 4 again, we find that colza oil exports which in which in the sixties had been in the range of 30 000 - 50 000 tonnes, rose to 130 000 and even 160 000 tonnes. In other words, the world market enabled us to sell the colza oil surpluses that accumulated as a result of declining demand on the Community market; this was achieved without difficulty and without subsidies. In this connection, I would like to conclude with a question which you may find somewhat provocative.

I wonder why a product which is not accepted by Europe's consumers is well received by consumers in other countries, to the extent that we export our own output and then import oils of foreign origin, the superior quality of which remains to be conclusively demonstrated. It is with this question that I will conclude my talk.

Table 1 : Comparison of colza figures in 1955/56 (1) and 1975/76 (2) 1. Oilseeds ('000 t)

	Germany		France		Italy		Netherlands		UEBL/BLEU		United Kingdom		Ireland		Denmark		EUR
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(2)
Total production	28	198	141	679	156	56	62	50	22	7		67		0	12	134	1 191
- of which colza	21	198	98	508	10	3	26	37		1		67		0	3	131	945
Net imports	1084	4533	761	588	157	1361	574	1885	185	888		1298		3	155	418	10 974
- of which colza	4	141	10	-77	3	16	-7	52		2		60		0		-108	86
Availabilities	1112	4731	902	1267	313	1417	636	1935	207	895		1365		3	167	552	12 165
- of which colza	25	339	108	431	13	19	19	89		3		127		0		23	1 031

2. Vegetable fats and oils (except olive oil)

	Germany		France		Italy		Netherlands		UEBL/BLEU		United Kingdom		Ireland		Denmark		EUR
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(2)
Total production	386	1256	395	479	99	346	226	466	72	174		297		2		118	3 138
- of which colza	11	129	45	186	5	8	7	33		1		51		0		3	411
Net imports	373	-176	123	344	112	292	5	-100	42	45		763		31		-24	1 175
- of which colza	7	-46	-4	-133	-2	25	-4	-27		8		5		2		-3	-169
Availabilities	759	1080	518	823	211	638	231	366	114	219		1060		33		94	4 313
- of which colza	18	83	41	53	3	33	3	6		9		56		2		0	242

Source : EUROSTAT

COLZA SEED PRODUCTION

Table 2

'000 tonnes

	Average 1934/38	Average 1948/53	Average 1952/56	1966	1976[1]
INDIA	745	823	914	1 276	1 400
CHINA	2 480	780	916	1 120	1 300
POLAND	48	100	100	448	983
FRANCE	13	154	119	317	561
CANADA	...	9	41	585	930
PAKISTAN	232	267	291	278	270
EAST GERMANY[2]	...	110	138	211	318
SWEDEN	...	146	120	95	244
WEST GERMANY[2]	...	83	33	99	222
JAPAN	119	129	276	95	6
WORLD TOTAL	3 890	2 800	3 150	4 835	6 990

Source: FAO

[1] Source: Oil Word
[2] German production in 1934/38 was 86 000 tonnes

Table 3

Production of colza and rape seed in the Community

Product	Member State	Production ('000 tonnes)									
		1967	1968	1969	1970	1971	1972	1973	1974	1975	1976
Colza and Rape	Germany	124.6	169.9	158.1	185.0	228.3	248.7	222.3	301.1	199.0	221.5
	France	432.5	457.5	512.5	502.4	649.7	713.0	659.6	672.7	507.6	560.9
	Italy	5.3	4.7	4.4	5.5	5.7	6.4	10.6	3.0	2.5	1.7
	Netherlands	15.2	18.0	12.2	21.8	32.7	45.2	40.6	44.9	36.6	34.2
	Belgium	1.2	1.6	1.1	1.2	1.5	2.9	2.1	1.0	0.7	0.4
	Luxembourg	-	-	-	-	-	-	-	-	-	-
	EEC 6	578.8	651.7	688.4	806.0	917.8	1016.2	935.2	1022.7	746.4	818.7
	United Kingdom					14	130.0*	30.8	55.4*	61.0	111.0
	Ireland					.	0	0	0	0	0
	Denmark				50.9	50.9	50.4	92.3	111.9	130.6	81.1
	EEC 9					972.9	1079.7	1058.3	1190.0	937.9	1010.8

Source : EUROSTAT

*Estimate by EEC Commission department (DG VI)

Table 4

Supplies of colza seed and derived products

Item	EUR 6							EUR 9			
	1967/68	1968/69	1969/70	1970/71	1971/72	1972/73	1973/74	1973/74	1974/75	1975/76	1976/77
1. Colza seed											
Seed production	579	652	688	806	918	1016	935	1058	1190	938	1023
Imports	241	210	109	455	495	284	378	386	167	150	599
Exports	51	31	66	79	35	44	54	46	220	59	46
Availabilities	769	831	731	1182	1398	1250	1259	1398	1137	1029	1376
2. Colza oil											
Colza oil production in the EEC											
- from Community seed	204	253	249	322	360	396	361	415	398	343	381
- from imported seed	106	84	36	182	205	116	159	158	68	59	156
Total production of colza oil	310	337	285	504	565	512	516	573	466	402	537
Imports of oil	33	35	10	4	7	16	10	11	9	4	14
Exports of oil	45	39	27	50	95	70	136	124	161	135	138
Variation in stocks	+ 31	+ 10	- 25	+ 30	- 11	- 20	0	+ 2	+ 3	- 5	+ 2
Quantity available	267	323	293	428	488	478	390	458	311	276	411
(a) Availabilities of veg. oils and fats in EEC excl. olive oil	2622	2798	2811	2934	2802	3284	2980	4172	3594	4258	4396
(b) Availabilities of colza oil as % of veg. oils and fats in EEC, excl. olive oil	10.2%	11.5%	10.4%	14.5%	17.4%	14.6%	13.1%	11 %	8.7%	6.5%	9.3%
(c) Indigenous colza oil production from EEC seed as % of veg. oil in EEC excl. olive oil	7.8%	9.0%	8.9%	10.9%	12.9%	12.1%	12.1%	10 %	11.1%	8.1%	8.7%

Source : EUROSTAT
EEC Commission, DG for Agriculture

Symposium on New Varieties of Colza

M.J. MORICE
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Orientation actuelle de la sélection du colza :
objectifs, résultats obtenus, perspectives d'avenir.

SUMMARY

Colza varieties providing an oil with a low erucic acid content have become general in most of the producer countries. The far-reaching change in the composition of the oil has been accompanied by a levelling-off and in some cases a slight drop in yield. The intensive selection undertaken on the new type of colza has given a new impulse to improvement and has provided varieties as productive as the old ones, and even more so. Another aim of current research into the quality of the oil is to reduce the linolenic acid content.

The elimination of sulphur-containing goitrogenic products in oilcake is also being pursued. This work has already produced the first so-called double zero varieties. These should, in the next few years, gradually replace varieties which are merely free of erucic acid and should provide oilcake which is competitive with soya cake.

The use of new selection methods based on recent discoveries in the floral biology of colza point to a fairly spectacular rise in productivity.

THE SELECTION OF NEW VARIETIES OF COLZA

Jacques Morice, INRA, France

Over the last few years there has been a complete change in the colza varieties grown in most of the producer countries. In France for example the Major variety, which gave satisfactory yields and was also satisfactory in its resistance to the serious disease Phoma lingam, was suddenly replaced in 1973 by the new Primor variety, which was almost unknown to the growers.

The reason for this sudden change was the desire of the authorities and the producers' associations to make available to processors and consumers colza oil that would be immune from the current bombardment of criticism, even though these criticisms were often excessive both in form and in substance. They wanted a rapid switch-over to varieties yielding an oil free of erucic acid, as this fatty acid was generally considered to be the cause of physiopathological diseases which could be induced in rats by colza oil.

The problem of oilcake quality is perhaps more real. The protein quality of colza oilcake is such that it could very largely take over from soya cake if it did not contain glucosinolates sulphur-containing products which hydrolize to give goitrogenic compounds. These limit its use and almost rule it out for those monogastric species (pigs and poultry) particularly sensitive to these compounds. The second varietal revolution is now taking place with the selection of colza varieties without glucosinolates. These are beginning to appear now and will become widely cultivated over the next few years.

Another research objective is increased yields and greater crop security through improved resistance to bad weather and pests.

I want to review the different aspects involved in the selection of new varieties of colza and will be mainly concerned with the quality aspect, which is a remarkable example of how genetics can remedy the nutritional defects of a crop product. I will illustrate what I have to say by examples from the work that has been done in France, not out of nationalism but simply because that is the work with which I am most familiar, having been in charge of genetic and improvement work on colza at INRA for more than twenty years. The objectives of the work done in other countries (in Canada and in Europe particularly in Germany, Great Britain, Poland and Sweden) are the same, and similar results are being obtained through programmes planned and carried out slightly differently.

IMPROVING THE QUALITY OF COLZA OIL

Colza oil is normally characterized by long carbon chain fatty mono-unsaturated acids, gadoleic acid with 20 carbon atoms and more especially erucic acid with 22 carbon atoms representing around 10 and 50% respectively of the total fatty acid content (Fig. 1).

1: COMPOSITION OF SOME EDIBLE OILS

(Fatty acids expressed as % of total)

Acid		Colza	Sunflower	Soya	Groundnut	Olive
Palmitic	C 16 : 0	2-4	5-8	10-12	6-8	8-12
Palmitoleic	C 16 : 1	0,5-1,5				
Stearic	C 18 : 0	1-2	2-5	2-4	3-6	1-3
Oleic	C 18 : 1	10-15	25-35	25-30	50-70	70-85
Linoleic	C 18 : 2	10-16	50-65	50-60	15-25	6-10
Linolenic	C 18 : 3	8-11		6-9		
Arachidic	C 20 : 0	0'5-1		0,5-1	3-5	1
Gadoleic	C 20 : 1	9-13				
Behenic	C 22 : 0				2-3	
Erucic	C 22 : 1	40-55				

Even before numerous nutritional studies made during the last few years had shown the consequences of ingestion of colza oil on various organs, particularly the cardiac muscle, in several animal species selection programmes had commenced in several countries with the aim of eliminating erucic acid, the presence of which already appeared undesirable.

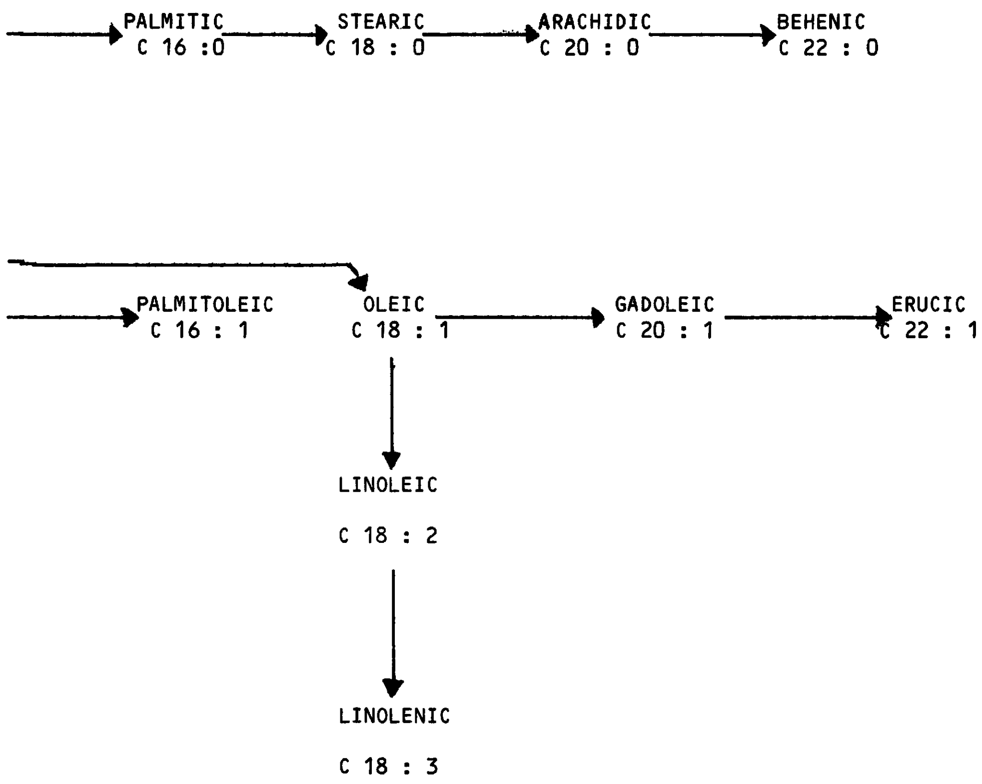
In 1961 Canadian researchers had isolated plants yielding an oil free of erucic acid. This discovery was the origin of the Canbra spring varieties. The Canadian geneticists generously supplied their precious material to European selectors, who used it widely as a genitor in hybridizations with European spring and winter varieties and lines.

In this colza the biosynthesis of the fatty monounsaturated acids is blocked at oleic acid, as the seed does not possess the enzyme systems for lengthening the carbon chain (fig. 2).

Genetic studies have shown that the hereditary determination of erucic acid content is simple. Two genes control the biosynthesis of gadoleic acid and erucic acid at seed level: the Canadian colza has a double recessive mutation. The selector's task is to introduce these two recessive genes into varieties suitable for commercial cultivation.

The modern varieties and lines are the product of several selection cycles which have built up a large number of favourable hereditary qualities. The

2. FATTY ACIDS IN COLZA OIL



The only desirable characteristic of the Canadian genitor was the lack of erucic acid in the oil: its yield was low and its cultural value poor.

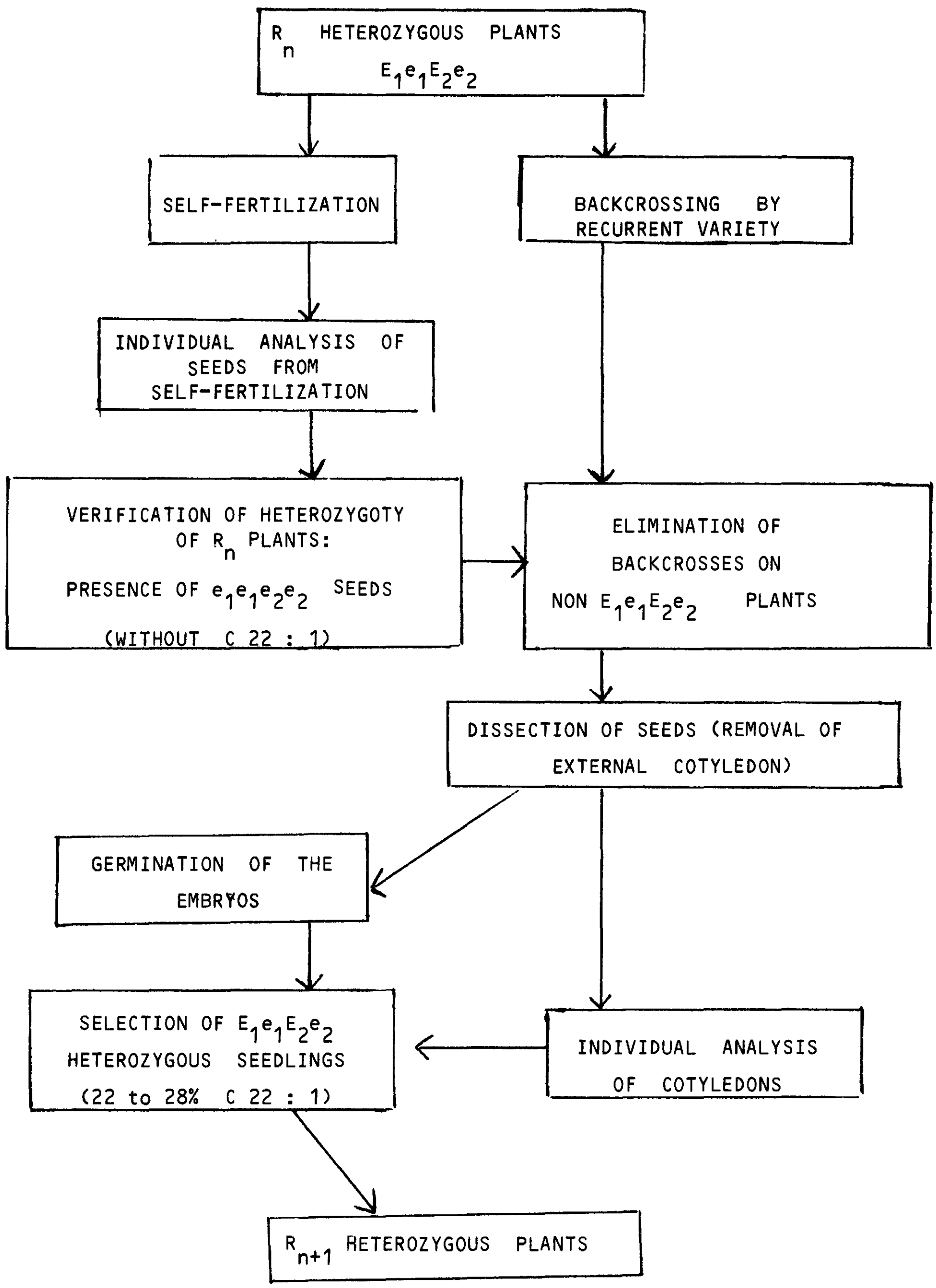
In our selection programmes we therefore set out to convert our best varieties by backcrossing. With this method, the laws of genetics after several generations cause all the characteristics of the variety used in the successive backcrossings to reappear with the exception of the characteristic that was the subject of the selection, in our case the erucic acid content. It is by this mehtod that we have produced the Primor variety in France, a form of the Major variety without erucic acid (fig. 3).

3. SELECTION OF THE PRIMOR VARIETY

Plant type and generation	Genes controlling the erucic acid	Genes controlling the other characteristics
PARENTS Canadian colza CANBRA Winter colza OLEOR	 $e_1e_1e_2e_2$ (0 % C 22 : 1) $E_1E_1E_2E_2$ (45 - 50 %)	 100% Canadian C spring 100% Winter H
Fl : CANBRA x OLEOR	$E_1e_1E_2e_2$ (25 - 30%)	50% C - 50% H
R_l : (first backcrossing) (C x O) x winter line	1/4 $E_1E_1E_2E_2$ (45 - 50%) 1/4 $E_1e_1E_2E_2$ (35 - 40%) 1/4 $E_1E_1E_2e_2$ (35 - 40%) 1/4 $E_1e_1E_2e_2$ (22 - 30%)	25% C - 75% H
R_2 : $E_1e_1E_2e_2$ plants from R_1 X MAJOR	same combinations as for R_1	12.5% C - 87.5% H of which 50% MAJOR
R_3 to R_7 $E_1e_1E_2e_2$ plants X MAJOR	same combinations	At R_7 : 0.4% C 99.6% H of which 97.4% M
Self-fertilization of $E_1e_1E_2e_2$ plants from R_7	1/16 $e_1e_1e_2e_2$ = PRIMOR 0% C 22 : 1	as R_7 97.4% M

With each generation great care must be taken not to lose the precious "Canadian" genes. This requires checking and selection as follows (fig. 4).

4: DIAGRAM OF SELECTION OPERATIONS FOR ERUCIC ACID AT EACH BACKCROSSING GENERATION



This is carried out at a very early stage on the actual embryo of the seed. It is possible to tell from the composition of the oil of one of its cotyledons what the genetic makeup of the plant will be.

Selection is accelerated by cultivation in air-conditioned chambers and under glass, which enables the vegetative cycle of the plant to be shortened. This is particularly important for winter colza, which in natural conditions takes roughly ten months from sowing to maturity. Artificial vernalization by keeping the young plants in cold chambers for several weeks followed by cultivation under glass (with additional lighting in winter) makes it possible to produce two generations per year.

Colza has the advantage of a high multiplication rate, which means that the results of research are available to the farmer fairly quickly. The example of Primor (fig. 5) shows that from 200 seeds obtained in 1971 roughly 2 000 tonnes of certified seed was produced in 1973, which yielded between 400 000 and 500 000 tonnes of colza seed free of erucic acid in the 1974 harvest.

The results of selection can be expressed in terms of both productivity and oil composition. Experimentation from 1972 to 1976 established an average yield for Primor around 5% below that of Major (fig. 6):

6. MAJOR AND PRIMOR YIELD COMPARISON

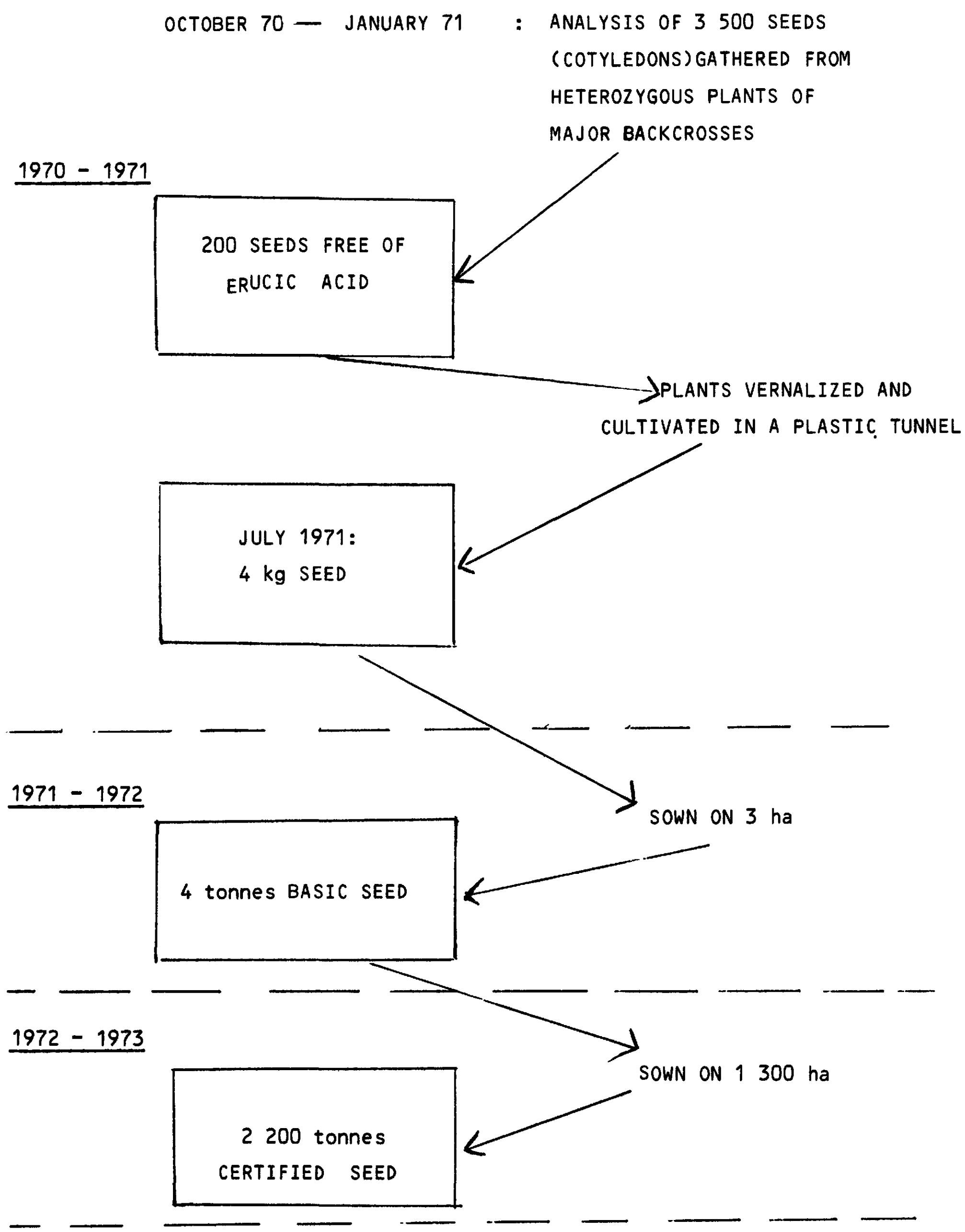
INRA and CETIOM comparative trials 1972 to 1976

YEAR	MAJOR	PRIMOR
1972 (4 trials)	26.20	25.00
1973 (22 trials)	26.45	25.80
1974 (36 trials)	24.95	23.80
1975 (23 trials)	25.20	23.20
1976 (14 trials)	24.15	23.15
Five-year average	25.39	24.19

The difference is not negligible, but for a first selection cycle for winter colza free from erucic acid the prototype obtained is not very far removed from the best variety of winter colza available at the time.

As regards the composition of the oil (fig. 7), erucic acid and gadoleic acid have almost completely disappeared resulting in a considerable increase in oleic acid content, a fairly big rise in linoleic acid content and fortunately little effect on linolenic acid content.

MULTIPLICATION OF SEEDS
OF THE PRIMOR VARIETY



7. COMPOSITION OF THE OILS OF MAJOR AND PRIMOR

ACID		MAJOR	PRIMOR
Palmitic	C 16 : 0	3.5	4.5
Palmitoleic	C 16 : 1	0.4	0.6
Stearic	C 18 : 0	1.2	1.5
Oleic	C 18 : 1	14.2	60.5
Linoleic	C 18 : 2	13.8	21.5
Linolenic	C 18 : 3	9.1	10.3
Gadoleic	C 20 : 1	10.9	0.9
Erucic	C 22 : 1	46.9	0.2

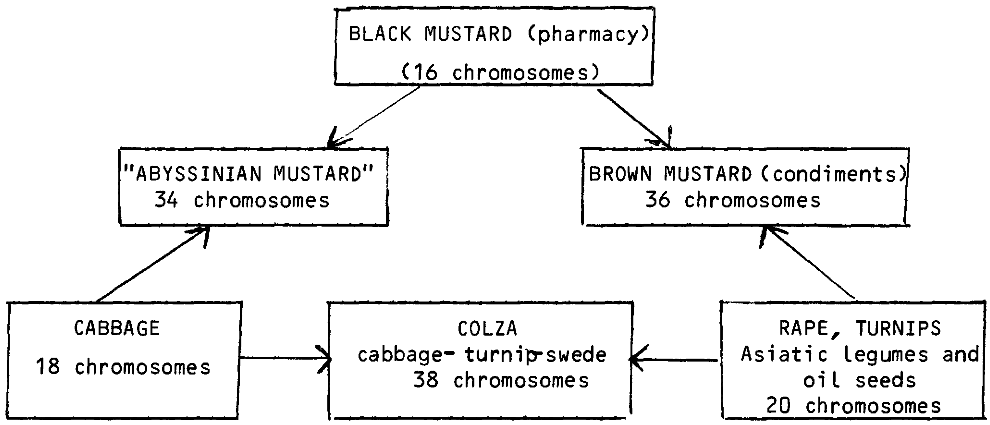
By slightly different methods using backcrossings alternating with selection generations our colleagues in Sweden and Germany have also obtained varieties without erucic acid the first of which have also yielded slightly less than the conventinnal varieties.

New non-erucic acid varieties with better yields are now appearing: in France for example the winter variety Jet Neuf (Ets Ringot), obtained by the same method as Primor but using the latter as a genitor instead of the Canbra spring variety, marks a spectacular step forward; apart from the fact that its health condition is better, its yield is around 120% that of Primor.

Another quality problem for colza oil is the relatively high linolenic acid content of around 10%. Linolenic acid oxidizes easily, especially if the temperature of the colza oil is raised, and this restricts its use to oils for salads and seasonings. This limitation is obligatory in France, where under current legislation the maximum linolenic acid content of oil for use in frying is 2%. For this reason and for nutritional reasons, although the conclusions here are not yet very clear, research has begun with the object of reducing the linolenic acid content by selection.

No varieties have as yet been obtained, as the selection possibilities for this characteristic are restricted. Besides exploitation of the apparently limited natural variability of colza. Work on linolenic acid has taken two other directions. The first is exploration of the variability of the parent species of colza: cabbage and rape (fig. 8). The seeds of these plants give an oil the normal composition of which is close to that of colza, but they afford much more in the way of variation and it has been possible to select lines of cabbage and rape whose oil has a linolenic acid content of below 5%. The work now consists of producing colzas by hybridization between the two species. This is an onerous undertaking requiring the use of fairly complex techniques (embryo culture "in vitro", cytological techniques, etc.) and is a fairly long-term project.

8 : GENETIC RELATIONSHIPS BETWEEN COLZA AND ITS RELATED SPECIES



The second method, more rapid if it succeeds, is to increase the mutation rate by the action of certain chemicals or radiations in the hope that the genes controlling the normal biosynthesis of linolenic acid will be affected by mutations. In this way, out of several thousand plants descended from Primor seeds treated with mutagenic agents, besides multiple mutations affecting morphological characteristics some plants have been discovered with linolenic acid contents below 6%.

IMPROVING THE QUALITY OF COLZA OILCAKE

Colza oilcake ought to be a choice protein feedingstuff (fig. 9). Its proteins are well balanced as to the proportion of amino-acids and the lysine content is close to that of soya cake (between 5.5 and 6% of the total amino-acids). Unfortunately it contains anti-nutritional substances which limit its use (fig. 10).

These are glucosinolates, sulphur-containing compounds which hydrolize in the presence of an enzyme, mysosinase, which is also present in colza seed, and give products which render the cake unappetitising and cause a malfunctioning of the thyroid gland, which hypertrophies, this being the reason why these products are referred to as "goitrogenic". The animals' growth is retarded in comparison with that of animals fed on soya cake. This disadvantage is much more serious in monogastric animals than in ruminants.

There are technological or biological methods for detoxifying the cake, but they are either only partially effective or too expensive for an animal feedingstuff.

9 : COMPOSITION OF THE PROTEINS OF COLZA AND SOYA
(Amino-acids expressed in g. per 16 g. of nitrogen)

Amino-acid	Colza samples[1]	Soya[2]
Glycine......	4.60 - 5.10	4.3 - 4.7
Alanine......	4.00 - 4.70	4.4 - 4.6
Valine.......	4.80 - 5.05	4.9 - 5.5
Leucine......	6.45 - 6.90	7.5 - 7.8
Isoleucine...	3.65 - 4.10	4.7 - 5.2
Serine.......	3.75 - 4.25	5.1 - 5.6
Threonine....	3.95 - 4.35	3.9 - 4.4
Tyrosine.....	2.65 - 2.95	2.9 - 3.9
Phenylalanine	3.70 - 3.80	4.9 - 5.2
Proline......	6.50 - 7.50	6.2 - 6.5
Trytophane...	0.90 - 1.10	1.0 - 1.3
Methionine...	1.95 - 2.15	1.4 - 1.6
Cystine......	2.50 - 2.60	1.5 - 1.7
Lysine.......	5.65 - 6.00	6.1 - 6.8
Histidine....	2.55 - 2.70	2.4 - 2.8
Arginine.....	5.95 - 6.35	6.9 - 8.4
Aspartic acid	6.40 - 7.05	11.5 - 12.4
Glutamic acid	17.85 - 18.35	20.4 - 21.1

[1] INRA samples analysed by the Versailles protein laboratory.

[2] Figures taken from various publications.

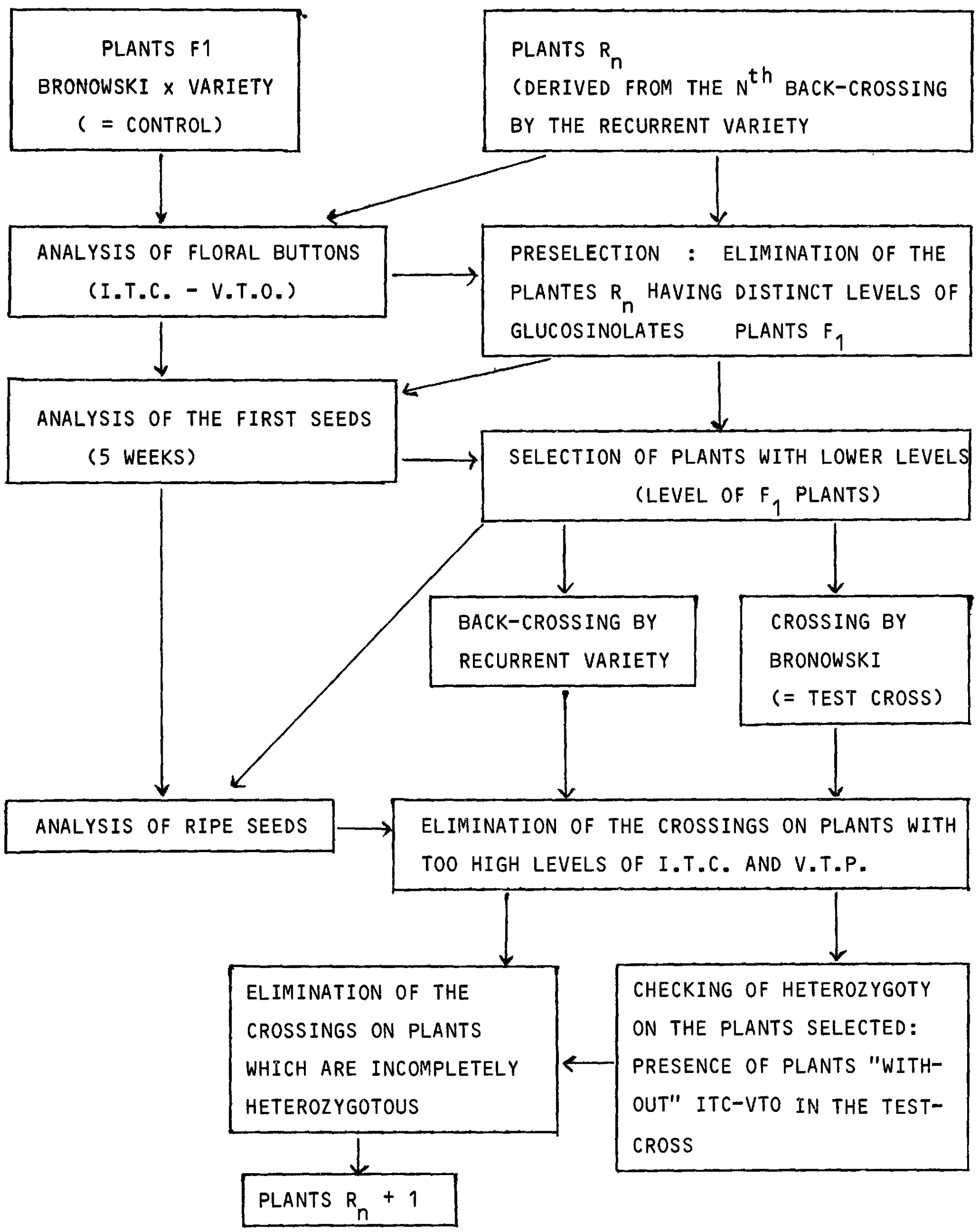
The selection of varieties without glucosinolates is certainly the best solution. Fortunately there is available in this connection a remarkable genitor, a Polish spring colza, Bronowski, which all the breeders have employed in their crossings. Various selection methods have been used and a number of spring varieties free of both erucic acid and glucosinolates have already been obtained, particularly in Canada where they are already being grown grown on a large scale. These are the colzas termed "double zero". In France we have initially concentrated our efforts on winter colza, which accounts for 90% of total production. Since the genetic differences between winter colza and the Bronowski genitor are much greater than those between spring colza and Bronowski it is more difficult and will take longer to produce strains, having a yield and quality equal to those of the best varieties, and incorporating the only interesting characteristic of Bronowski, its very low glucosinolate content.

10 : MAIN SULPHUR-CONTAINING PRODUCTS OF CRUCIFERS AND THEIR PROPERTIES

Sulphur-containing products	Hydrolysis products	Physiopathological effects		Organo-leptic properties
		Hyper-thyroidism	Anaemia	
Sinigrin	Allyl I.T.C.	+		++
Gluconapin	Butenyl I.T.C.	+		++
Progoitrin	V.T.O.	++		
Glucobrassicanapin	Pentenyl I.T.C.	+		
Glucobrassicin	Thiocyanate T.C. H_2S	+		+
Methyleysteine	Methyl trisulphides			
Sulphoxide M.C.S.	T.S.M.		++	

In France we have undertaken selection by back-crossing after initial hybridization between Bronowski and our better varieties of colza. The greater complexity of the hereditary determinism and the fact that the biosynthesis of glucosinolates is controlled at the plant level for all its seeds, and not at the level of each embryo on one plant, necessitated the use of a complex series of operations and chemical analyses for each generation (Fig. 11). The selection, as in the case of erucic acid, is carried out under glass to speed up the generation rhythm. It started with the variety Major as the recurrent genitor and finished up with a form containing glucosinolates, one family of which is at present being tested while another is under multiplication. Primor has been grafted into the programme of back-crossings and the first double-zero plants of the Major-Primor type are at present produced under glass. Similar work is being carried out at INRA on other lines, in particular the variety Jet Neuf. Taking into account the years necessary for the multiplication of the seeds and the official experiments on the first varieties, "double zero" winter colza should come into cultivation in 1982.

SCHEME OF OPERATIONS FOR SELECTION
FOR GLUCOSINOLATE AT EACH GENERATION
OF BACK-CROSSING



The production of colza without glucosinolates should make possible the introduction of oilcakes into the feeding of pigs and poultry in considerable quantities. The relatively high level of cellulose in these oilcakes (12-13%) is a defect which is particularly significant for these animals.

Two ways exist for remedying this defect; a technological way and a genetic way. The genetic solution has been tried out in Canada by the selection of spring varieties of rape and colza with yellow seeds, the thinner coats of which add less cellulose to the defatted oilcake. The difference may be as much as 4%. There is thus a promising result here but it applies more especially to rape. For colza the results obtained up to now are less promising.

The technological solution lies in the removal of the colza seed coating. Various procedures have been put forward in several countries. In France, a procedure has reached the industrial development stage. It enables the level of cellulose to be reduced from 12 to 6% (Table 12)

12 : COMPOSITION OF THE OILCAKES IN PERCENTAGE DRY MATTER

(CETIOM results)

	Normal Colza	Colza with seed coating removed	Soya 44
Cellulose	12,1	5,9	7,6
Proteins (N x 6,25)	37,7	51,7	60,2
Minerals	7,9	7,1	6,6
Non-nitrogen extract	40,6	33,6	33,9
Fats and oils	1,7	1,7	1,7

The analytical results and the tests carried out on animals show that the removal of seed coatings from colza without glucosinolates should give an oilcake which in both its energy and its protein value is similar to soya 44 oilcake.

IMPROVEMENT OF PRODUCTIVITY

Effective selection for the improvement of yields calls for a rational selection selection method to find the type of variety most suited for realising the plant's production potential. The mentod will depend upon the biology of the plant, its mode of reproduction, its reaction to forced self-fertilization, the effects of hybridization, etc.

Up to now, according to their interpretations of these phenomena, the selectors of colza have tended towards different methods of imrpvement leading to different varietal types.

Some of them have created variety populations with a fairly complex genetic structure to ensure both a flexibility of variety and a better yield level on the basis of a hybrid vigour brought about effecting crossings between yield level on the basis of a hybrid vigour brought about by effecting crossings between the individual lines composing the variety. This is the case generally with the Swedish and German varieties. In France efforts are being made to create pure lines, it being thought possible to select very good, stable and homogeneous homozygotous lines. In the United Kingdom this idea of homozygoty has been pushed further since varieties are being created there by spontaneous doubling of haploids.

These differences of opinion on such a subject may seem rather academic. They do, however, have practical consequences, some of which, of an administrative and regulatory nature, may give rise to some difficulties for the Joint Catalogue of varieties, since not all Community countries have the same criteria of homogeneity for their respective national Catalogues.

Whatever the methods of selection adopted, we have seen a gradual improvement in yields during the last twenty years in the various countries producing colza. This is true for France (Table 13) where, after an increase of more than 20% in 10 years and a slight fall at the time of conversion to colza without erucic acid, a new jump forward in productivity has been brought about.

13 : INCREASE IN YIELDS BROUGHT ABOUT BY THE SELECTION OF NEW VARIETIES FROM 1966 to 1977

(Average of 240 tests CETIOM and INRA over 8 years : 1969-1977)

Varieties	Year of entry in the Catalogue	% yields of Sarepta
SAREPTA	1962	100
TITUS	1966	104,7
MARCUS	1969	106,4
RAMSES	1970	108,2
MAJOR	1971	121,2
PROMOR	1973	116,0
JET NEUF	1977	136,0

The results of recent studies may lead to a change in colza selection policy, in favour of F_1 hybrids, a type of variety which enables the potentialities of the species to be exploited to a maximum.

It is necessary first to be able to produce the hybrid seed. For this purpose one must have available genetic mechanisms making the colza line female and preventing it from self-fertilization with its own pollen. Current research on autoincompatibility and on male sterility carried out by several teams in Europea and Japan has advanced the knowledge of these phenomena.

At the Plant Improvement Station at Rennes, for example, for several years various types of cytoplasmic male sterility have been studied in order to render at least one of them operational in a programme for the production of hybrid colzas.

Parallel to this work on male sterility attempts are being made to calculate the improvement in yield which may be contributed by F_1 hybrids compared with the existing varieties. The results obtained in comparative trials over three years are fairly productive (Table 14). In comparison with the variety Primor regarded as a control, and appearing too as a parent in all the hybrid varieties tested, very definite increases in yield were observed.

14 : COMPARISON OF HYBRIDS - WINTER COLZA LINES

RENNES 1975 - 1976 - 1977

	1975	1976	1977
NUMBER OF HYBRIDS STUDIED	20	35	7
AVERAGE YIELD OF PRIMOR	100	100	100
AVERAGE YIELD OF LINES	85,7	87,9	81,5
VARIATION OF YIELD OF LINES	69,4 - 113,4	48,2 - 114,2	61,0 - 105,1
AVERAGE YIELD OF HYBRIDS	143,2	128,4	136,4
VARIATION OF YIELD OF LINES	122,5 - 171,2	107,1 - 154,7	102,0 - 147,0

Besides the ability to give a high yield, the productivity of colza calls into play a number of factors which contribute to the resistance to bad weather and pests. In the French selection programmes for winter colza the accent is placed mainly on resistance to cold, resistance to lodging, a certain precocity and resistance to Phoma lingain, a cryptogram disease which may cause considerable losses. The most recent work concerns resistance to another disease due to Sclerotinia.

CONCLUSIONS

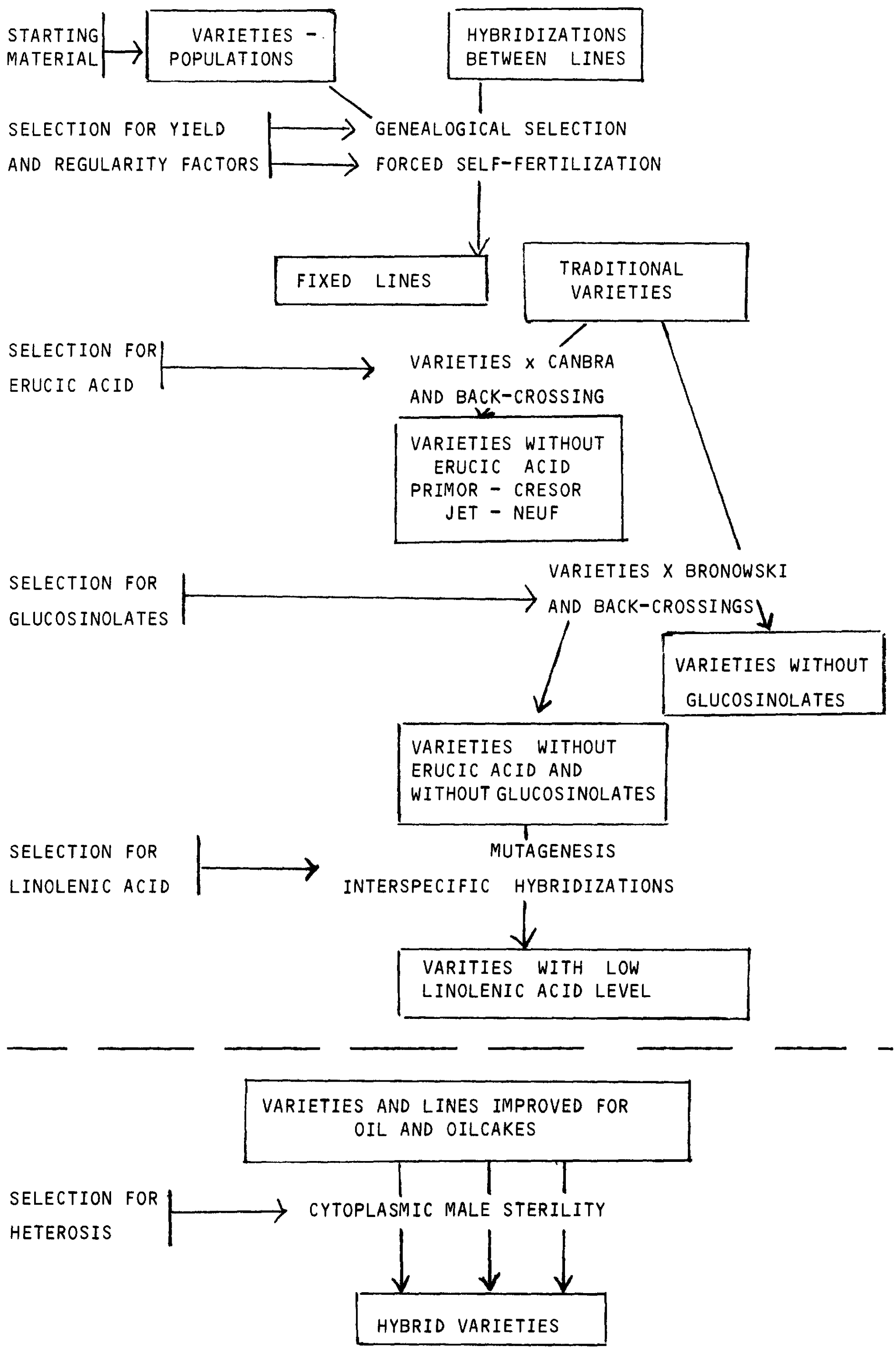
The problems of quality which have been added to the agronomic objectives of selection have led for some fifteen years to a large development and a wide diversification of research into colza. Table 15 which traces the stages of French selection work might, with a little variation and maybe some additional research, apply equally to other countries.

The efforts which have been made in the area of genetic improvement were justified by the agronomic and economic interest of this oil and protein-bearing plant. The successes already obtained, the achievements looked for from certain programmes, and the hopes placed in others, make of colza a remarkable example of the profound changes which genetics can bring about in the normal biology of a plant in order to adapt it qualitatively and quantitatively to the needs of man.

Colza has proved to be relatively flexible, offering to the selector sufficient variability in the form of rare mutants for its transformation. But all the research programmes performed or being carried out are fairly onerous and require considerable means. Clearly they can only be put in hand if the objectives are well defined. This is relatively simple as regards the agronomic aspects, since it is obviously desirable that the yield should increase and that there should be better resistance by the plant to its predators in terms of the damage they can do. It is not always so clear-cut as regards the quality aspects, and particularly where nutritional characteristics are involved. It is necessary in this field that our colleagues who are specialists in physiology and animal and human nutrition fix objectives for us which, without seeking to create an ideal food from the products of one plant, aim at least to eliminate from it the factors which are known with certainty to be harmful. It is to be hoped that the important international work done to create varieties without erucic acid will not have been devoid of value and that the elimination of glucosinolates will make a better oilcake. It seems now that, as regards the oil, it will not be necessary to try to select out the possibly undesirable factor of the insaponifiable fraction. What is perhaps needed is for the nutrition experts to fix for us an acceptable level of linolenic acid.

Selection provides new varieties for agriculture which organizes itself to produce the seeds rapidly and put them into cultivation. The farming organizations play on quality, not only to meet the wishes of the scientific authorities concerned with nutritional problems but also in the hope of increasing the outlets for their production. The consumers should be made aware of the attention which has been paid to these problems and the efforts which have been made to provide them with an edible oil and an oilcake of good quality and they should be invited to take an objective look at the new products which are offered to them. I believe that the addresses by the specialists who are due to follow me will provide them with valuable information in this respect.

THE SELECTION OF COLZA IN FRANCE, STAGES ACCOMPLISHED, PRESENT SITUATION, PROSPECTS



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Exposé du Professeur J.K.G. Kramer

Low Erucic Acid Rapeseed Oil - A Quality Vegetable Oil

travaux de

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Low Erucic Acid Rapeseed Oil - A Quality Vegetable Oil

J.K.G. KRAMER, H.W. HULAN, S. MAHADEVAN, F.D. SAUER, H.L. TRENHOLM and A.H. CORNER

Summary

The nutritional properties of low erucic rapeseed (LEAR) oils were extensively investigated at Agriculture Canada. Rats, pigs and monkeys were used as test animals to evaluate the nutritional, biochemical and pathological properties of LEAR oils compared to control oils such as corn, soybean, olive and safflower oils. Animals fed LEAR oils showed no depressed growth rate or early death, did not succumb to cold stress, showed no evidence of impaired mitochondrial activity and no cardiac lipidosis or free fatty acid accumulation. Monkeys and pigs showed no evidence of myocardial necrosis and fibrosis. In rats, however, a higher incidence of heart lesions was observed by feeding diets containing LEAR oils compared to control oils. The lesions in rats was dependent on: sex (male $>$ female), androgens (castration of males reduces the incidence), fat in the diet (incidence increases with increasing fat content in the diet), and the strain of rat (albino $>$ hooded). The cause of heart lesions is discussed. Erucic acid per se is not the sole cause of heart lesions in rats, because there is no direct relationship between erucic acid in the diet ($<$7%) and myocardial lesions. All attempts have failed to isolate, concentrate or remove cardiotoxic contaminants from LEAR oils. However, the scientific evidence to date supports a fatty acid imbalance as the cause of heart lesions in the fast growing albino male rat.

Exposé

In 1960 the first report appeared in the literature which indicated that feeding rapeseed oil to rats was associated with myocardial pathogenesis (47). This problem was believed to be caused by erucic acid. Plant breeders successfully met the challenge of removing most of this fatty acid from the oil. However, it became apparent from the results of Rocquelin (45) that a high incidence of heart lesions still persisted with the low erucic acid rapeseed oils.

Since Canada is a major producer and exporter of rapeseed oil, Agriculture Canada decided in 1971 to form a research team to investigate the properties of low erucic acid rapeseed oils. The team consisted of members from various disciplines (Table 1).

Table 1. Agriculture Canada Research Team

Pathologists:	K.M. Charlton	Nutritionists:	H.W. Hulan
	A.H. Corner		D.W. Friend
	F. Gilka	Physiologist:	W.G. Hunsaker
Biochemists:	D.S. Dow-Walsh	Statistician:	B. Thompson
	S. Mahadevan	Toxicologist:	H.L. Trenholm
	F.D. Sauer	Lipid Chemist:	J.K.G. Kramer

We were assigned the task of assessing the problems ascribed to rapeseed oils in general and more specifically to low erucic acid rapeseed oils. The talk will be discussed under the following headings: firstly, defining the nature of the problem; secondly, to give the results of comparative studies carried out with monkeys, pigs and rats to show the extent of these problems; and finally, evidence will be presented to explain the etiology of heart lesions in male rats.

Before proceeding, let me define abbreviations I will be using: HEAR = high erucic acid rapeseed; LEAR = low erucic acid rapeseed with less than 5% erucic acid. Reference to HEAR oils will only be made for comparison purposes.

First let us briefly examine the problem ascribed to rapeseed oil and determine whether these problems still occur with LEAR oils.

Weight gain. Earlier studies with HEAR oil demonstrated depressed growth rate which was attributed to the presence of 22:1 (50). No depressed growth rate has been observed in rats, pigs and monkeys fed LEAR oils compared to those fed diets containing control oils.

Longevity. In the longest feeding trial reported to date, 18 months, there were no significant differences ($P < 0.05$) in mortality of Fisher male and female rats fed 20% dietary levels of soybean oil, HEAR oil or crambe seed oil (16).

Cold Stress. It was reported that rats fed rapeseed oil succumbed to cold stress more readily than those fed corn oil (7). In fact, 87% mortality was reported in 6 week old rats kept at 4°C and fed a HEAR oil for 3 weeks (11). In two repeat studies carried out for 4 weeks under identical conditions, we could not confirm the high mortality with HEAR oil. Rats fed LEAR oils under the same circumstances showed low mortality similar to rats fed control oils.

Heart mitochondria. Mitochondria isolated from the hearts of rats fed HEAR oil were reported to show impaired respiratory activity, and partial loss of respiratory control (27). This report was questioned since we showed that properly isolated mitochondria from rapeseed oil-fed rats are functionally intact with respect to oxidation and energy-coupling capacity (19,41). Several investigators have recently confirmed the functional integrity of heart mitochondria from rapeseed oil-fed rats (9, 17, 25).

Myocardial lipidosis. The accumulation of lipid droplets in heart muscle fibres is a finding observed under several pathological conditions such as stress, hypoxia and ethanol intoxications (20). Myocardial lipid accumulation was demonstrated shortly after feeding HEAR oils in male and female rats (1, 14, 20, 41, 46), male and female ducklings (2), male and female chicks (35, 42), boars and gilts (10, 34, 49, 52), and in male and female monkeys (4, 26). The extent and response of myocardial lipidosis varies between species, age, the younger reacting more severely (10) but male and female are affected equally.

In contrast, myocardial lipidosis is either mild or not detected at all when LEAR oils were fed to male and female rats (3, 14, 20, 41, 46), male chicks (35, 42), boars and gilts (10, 22), and male and female monkeys (39). Lipidosis is directly related to the level of 22:1 in the diet (3, 13, 4, 1). The longer long-chain monoenes cause more severe lipidosis (12). Several groups have attempted to determine a "zero-effect" level of 22:1 (3, 20). The 1% level of erucic acid in the diet, which represents a 5% level of this acid in the oil fed at 20% by weight of the diet, was considered a reasonable no effect level (20).

Changes in cardiac neutral lipids. Myocardial lipidosis was reported to be associated with triglyceride and free fatty acid accumulation (27).

A study (36) was undertaken to investigate triglyceride and free fatty acid levels in rats fed soybean oil, LEAR oil and HEAR oil (Table 2). LEAR oil elevated triglyceride levels slightly at 1 week only; free fatty acid levels remained similar to control. HEAR Oil caused a large triglyceride accumulation which decreased to 2 times control levels at 16 weeks; free fatty acid levels showed a temporary accumulation within the first week.

Table 2. Cardiac triglycerides (TG) and free fatty acids (FFA) in male Sprague-Dawley rats fed experimental diets (36).

Diet (% 22:1)	Time on diet (days)	TG (μg/g)	FFA (μg/g)
Soybean oil	3	2,000	95
	7	2,000	113
	112	1,700	47
LEAR (0.2%)	3	1,400	73
	7	3,200	98
	112	1,500	46
HEAR (22.3%)	3	14,400	210
	7	29,300	210
	112	4,500	68

It is evident that all the aforementioned problems do not occur with LEAR oils. We will next examine the myocardial necrosis and fibrosis reported in male rats fed HEAR, as well as, LEAR oils.

Myocardial lesions of this type are reported to occur on continued feedings of rapeseed oils to male rats for at least 12 weeks.

Let us look at the scope of this problem by examining the results of different animals species: Monkeys, pigs and rats.

Table 3. Cynomolgus monkeys fed diets which contained soybean oil or Tower RSO for up to 24 weeks (39,40).

Diet	Sex	Affected/Examined
Soybean oil	Male	2/7
	Female	0/7
Tower RSO	Male	0/14
	Female	0/12

The only study to date (Table 3) where monkeys were fed a LEAR oil was conducted under contract at Montreal (39,40). Two of the males fed soybean oil had degenerative cardiac muscle cell alterations which were surrounded by mononuclear cellular infiltrates. These are not the severe necrotic lesions observed in male rats. None of the 26 monkeys fed Tower RSO had heart lesions.

Table 4. Myocardial lesions in pigs fed diets containing no fat, control oils and rapeseed oils. Summary of 6 experiments.

Strains	No. of control diets	RSO Diets % 22:1	No. Sex	Time on Diet (wks.)	Affected/Examined * Control (%)		RSO (%)		Ref.
Yorkshire	2	4	96M,96F	1-16	34/120	(28)	22/72	(31)	22
Crossbred	2	4, 21	56M,56F	16	no difference				6
Yorkshire	2	22	72M	16	2/48	(4)	3/24	(13)	23
Norwegian Landrace	1	44	40 (M&F)	1-52	no difference		10/40	(25)	49
Crossbred	1	0.3, 1.2 4.9, 34	40M,40F	4-23	3/16	(19)	2/64	(3)	5
Yorkshire	2	1, 24	60M	24	20/30	(67)	22/30	(73)	24

*The lesions found are minute focal interstitial infiltration of mononuclear cells and not overt myocardial necrosis as found in rats.

The results of experiments evaluating rapeseed oils using pigs as test animals are summarized in Table 4. Six large studies were reported where a total of 556 boars and gilts were examined (5, 6, 22, 24, 49). The results indicated: (a) there were no significant differences in the incidence of heart lesions between controls and those fed any of the rapeseed oils, (b) no significant difference between sexes, (c) lesions found in most pigs were minute focal interstitial infiltrations of mononuclear cells, and not foci of overt myocardial necrosis as seen in male rats. In fact, it has been suggested by the University of Alberta researchers (5, 6) that Ascaris suum larvae migration might have been involved in the etiology of the lesions in the pig.

Now let us consider the results available from rats. At the outset it must be remembered that cardiovascular damage in growing rodents related to diet is a common finding. Rings and Wagner (44) referred to 13 possible dietary alterations that produce myocardial necrosis and mineralization in rodents. In fact, all attempts have failed so far to find a rat and design a diet which would give consistently a zero incidence of myocardial lesions. The use of a greater number of rats per treatment (33) or an exhaustive sectioning of each heart sample (51) have always confirmed a relatively high incidence of heart lesions in all groups fed control diets. This has led some investigators to designate the background incidence of myocardial necrosis and fibrosis in rats as "spontaneous" (3), or "normal" (21).

However, the fact remains, heart lesions are found by feeding any control oil, and a high intake of rapeseed oils only exacerbates something which is present in any case (28, 30, 32, 33, 38, 41).

Table 5. Summary of Agriculture Canada data on male rats fed diets containing 20% by weight fat or oil in the diet for 16 weeks.

Diet (% 22:1)	Affected/Examined	%	No. of expts.
Lard	14/75	19%	2
Corn oil	71/233	30%	7
Olive oil	38/120	32%	2
Safflower oil	7/20	35%	1
Soybean oil	84/181	46%	3
Span RSO (4.5%)	103/159	65%	7
Zephyr RSO (0.9%)	117/161	73%	6
Tower RSO (0.2%)	154/220	70%	6

It has been well established that the myocardial lesions discussed here occurred primarily in male rats and at much lower frequency and severity in female rats as seen in Table 6 (30, 41, 45, 53).

Increasing the level of corn oil in the diet of rats from 5% to 20%, significantly increases the incidence of heart lesions (28, 30). This was demonstrated in 2 separate experiments (Tables 6 and 7), and I believe the results of Rocquelin confirm this finding.

Table 6. Myocardial lesion incidence in Sprague-Dawley male rats fed experimental diets for 16 weeks. Effect of sex (30).

Diet (% 22:1)	Male	Female
5% Corn oil	3/26	0/26
20% Corn oil	7/26	4/26
20% Zephyr RSO (0.9%)	16/25	3/26

X^2 Analysis: male vs. female, $P < 0.001$
5% vs. 20% corn oil, $P < 0.05$

Table 7. Myocardial lesion incidence in albino male rats fed experimental diets for 16 weeks (28).

Diet (% 22:1)	Affected/Examined
5% Corn oil	6/101
20% Corn oil	23/99
20% Zephyr RSO (0.9%)	65/96

X^2 Analysis: 5% vs. 20% Corn oil, P 0.001
Zephyr RSO vs. Corn oil, P 0.001

Now having established the fact that myocardial lesions are found primarily in the male rat fed diets rich in fat, let us consider the cause of the higher incidence of heart lesions in male rats fed rapeseed oils. Basically three hypotheses have been formulated: (a) long-chain monoenes 20:1, 22:1 and 24:1, (b) toxic contaminant(s) present in rapeseed oils, and (c) fatty acid imbalance.

Let us consider the first hypothesis that long-chain monoenes are causing the heart lesions. The long-chain monoenes, comprising as little as 0.2 to 0.5% 22:1 in many of the new LEAR oils, are believed to be responsible for the increased incidence and severity of cardiac lesions. The main proof of this hypothesis was the ability to reproduce the heart lesions by feeding a trierucin-sunflower oil mixture, an oil which had an approximate erucic acid level of 42% (3). Canadian workers were also able to produce these lesions by interesterifying 30% 22:1 with olive oil (8). These results looked convincing by themselves, but remember that in order to produce these lesions, a very high level of 22:1 is required, 30 (8) and 42% (3).

There is strong evidence to indicate that 22:1 *per se* at levels of less than 5% is not the sole cause of myocardial lesions. The addition of 5.6% free erucic acid to lard (32) and 5.7% to soybean oil (29) did not increase the incidence of lesions; nor did the addition of 0.6 and 5.7% 22:1 to Tower RSO (29) have any effect.

Erucic acid present in the esterified form likewise did not significantly increase the incidence of heart lesions. Rendered pig fat which contained 5.6% 22:1 had a lesion response similar to controls (32). A simulated HEAR oil containing 28.7% 22:1 but no 18.3 was comparable in lesion incidence to soybean oil (43). Hydrogenation of LEAR oil to IV 77 did not affect the 22:1 level significantly but this process significantly lowered the incidence of heart lesions (15, 48).

Mixing of HEAR oils with non-Brassica oils lowered the incidence to control levels while the 22:1 still remain very high (3, 13).

This evidence renders the 22:1 toxicity hypothesis unlikely.

Table 8. Myocardial lesions in Sprague-Dawley rats fed Span RSO and its molecular distillation (MD) fractions (37, 38).

Diet	% 22:1	% Sterol	Incidence
Span RSO	4.8	0.25	23/50
Span RSO - MD5	3.1	0.01	31/50
- MD6	4.5	0.03	24/50

Table 9. Myocardial lesions in Sprague-Dawley rats fed Span RSO and its adsorption chromatography (AC) fractions (37, 38).

Span RSO	4.8	0.25	17/20
Span RSO - AC F1	8.8	0.62	19/20
- AC F2	6.2	N.D.	16/20
- AC F3	4.2	0.006	16/20
Span RSO	4.8	0.25	6/20
Span RSO - AC F2	6.2	N.D.	8/20

X^2 Analysis: Span RSO vs. AC fractions, NS.

The hypothesis of a toxic component was first put forth by Rocquelin et al (45) because LEAR oils gave similar results to HEAR oils. We considered this possibility and accordingly fractionated 22:1 by molecular distillation Span rapeseed oil containing 4.8% (37,38). Molecular distillation is an efficient distillation process of high molecular weight compounds such as triglycerides, which comprise 96% of the oil. Highly purified triglyceride fractions were prepared by this method (37). The low sterol content in these fractions, a component most difficult to remove from the oil was evidence of the purity of these triglycerides. The pathological results show (Table 8) that we were unable to remove any toxic contaminant since the incidence of heart lesions of the original oil was not significantly different from molecular distillates 5 and 6 (38). We also fractionated Span rapeseed oil by adsorption chromatography through large columns at tremendous costs to obtain highly purified triglycerides free of sterols (37). Again, no pathological differences (Table 9) were observed between the original oil and any of the fractions (38). These results clearly demonstrate that no so-called toxic component could be removed from rapeseed oil.

The fractionation was repeated using Tower RSO which contained only 0.2% 22:1. In the repeat study molecular distillation and adsorption chromatography were used in series, i.e. the pure triglyceride fraction from molecular distillation (Tower MD7) was further purified by adsorption chromatography. Two pure triglyceride fractions from the adsorption chromatography were tested. Soybean oil was used as the control oil. A similar molecular distillate of soybean oil was also tested. The pathological results confirm our previous findings that a toxic compound could not be removed from a LEAR oil; all fractions tested had a high incidence of heart lesions.

The two fractionation studies render the hypothesis that rapeseed oils contain toxic compounds highly unlikely.

We suggest a third hypothesis: the observed myocardial lesions in rats fed rapeseed oil may be related to the fact that the fatty acids present in rapeseed oil fail to meet the specific fatty acid requirements of the fast growing albino male rat (29, 32, 38, 41). To demonstrate this, we selected data from 3 independent laboratories: Agriculture Canada, Health and Welfare Canada and Unilever, the Netherlands. Twenty experiments in all were selected, which involved over 2000 3-week-old albino male rats fed for 16 or 24 weeks, diets containing less than 7% 22:1.

The main statistical technique applied in the study was a step-wise multiple regression analysis. The technique enabled us to explore possible relationships between heart lesions and the relative concentrations of dietary fatty acids. From the analysis we could demonstrate that the incidence of heart lesions was negatively correlated to the level of saturated fatty acids. In other words, test oils with low levels of saturates are associated with high lesion incidence and vice versa. Secondly, the heart lesion incidence was positively correlated to the level of 18:3 in test oils. The variation between experiments accounted for 29% of the variation, saturates for 40%, 18:3 for 6%, and all remaining fatty acids including 22:1 for 1%. A total of 77% of the variation was thus accounted for by the levels of dietary fatty acids.

A plot of observed incidence of heart lesions in male rats vs. incidence of heart lesions predicted by the regression equation is shown in Figure 1. It should be noted that, the relationship holds satisfactorily over the entire range. The results of all three laboratories (Figure 1) show a remarkably similar distribution around the regression line.

From Figure 2, it is evident that most of the upper points represent LEAR oils, while points in the lower range represent control oils. Please note that the points from control oils and LEAR oils from a continuum of points along the regression line with quite a region of overlap.

For the past decade the major concern of LEAR oils has focused on myocardial pathology of young growing male rats. The data suggest (Figure 1) that the pathological assay used is really a measure of fatty acid imbalance of the test oil and not an indicator of toxic contaminants or 22:1 in rapeseed oils. Since rapeseed oils in general are low in saturates, they give a higher incidence of heart lesions in male albino rats, because of the apparent requirement of higher levels of saturates in a high fat diet.

Please remember we are discussing hear a balance of dietary fatty acids to the albino male rate fed a high fat diet. Increasing the level of saturates in LEAR oil to reduce the incidence of heart lesions in this specific species, should not be considered a general recommendation to other species or man. For example, rapeseed oil appears to meet the fatty acid requirements of the pig and monkey, since no lesion difference was observed between LEAR and control oils in these two species. Secondly, we are well aware that increasing the level of saturates in the diet is not a desired alternative to man because of the known deleterious effects of diets rich in saturates.

In summary, LEAR oils are nutritionally safe for monkeys and pigs. Male albino rats, however, show a higher incidence of heart lesions than male rats fed control oils. There is evidence to indicate that the lesion incidence in male albino rats is directly related to the fat content in the diet. Secondly, the assay of heart lesions in male rats fed 20% by weight fat in the diet is not a measure of toxic contaminants or erucic acid, but really an assay of low saturates and the presence of 18:3 in test fats and oils.

Agriculture Canada Research Team

Pathologists:	K.M. Charlton	Nutritionists:	H.W. Hulan
	A.H. Corner		D.W. Friend
	F. Gilka		
		Physiologist:	W.G. Hunsaker
Biochemists:	D.S. Dow-Walsh	Statistician:	B. Thompson
	S. Mahadevan	Toxicologist:	H.L. Trenholm
	F.D. Sauer	Lipid Chemist:	J.K.G. Kramer

Problem definition

- examination of problems ascribed to rapeseed oils
- do these problems apply to LEAR oils

To assess the problems ascribed to rapeseed oils in general and whether these apply to low erucic acid rapeseed oils.

1. Problem definition
2. Comparative studies - monkey, pig, rat
3. Etiology of pathogenesis in male rats

Abbreviations

HEAR - high erucic acid rapeseed oil

LEAR - low erucic acid rapeseed oil

- erucic acid content < 5

WEIGHT GAIN

<u>High erucic acid rapeseed oil (HEAR)</u>

- depressed growth rate
    - in rats (1)
    - in pigs (2)
- attributed to 22:1 (1)

<u>Low erucic acid rapeseed oil (LEAR)</u>

- no depressed growth rate
    - in rats (3)
    - in pigs (4)
    - in monkeys (5)

1. Thomasson and Boldingh, J. Nutr. 56, 469 (1955).
2. Friend et al., Can. J. Anim. Sc. 55, 571 (1975).
3. Rocquelin and Cluzan, Ann. Biol. Anim. Biochem. Biophys. 8, 395 (1968).
4. Friend et al., Can. J. Anim. Sc. 55, 49 (1975).
5. Bio-Research study on primates conducted for the RUAP, 1975.

LONGEVITY

Male and female rats fed vegetable oils (20% by weight) for 18 months.

Rapeseed oil = Soybean oil = Crambe seed oil (1, 2).

1. Booth *et al.*, AOCS *49*, 304A (1972).
2. Tallent, W.H., private communication.

COLD STRESS

HEAR - 6 WEEK OLD RATS KEPT AT 4°C HAD 87% MORTALITY AFTER 3 WEEKS (1, 2).
- IN REPEAT STUDIES, NO MORTALITY AFTER 4 WEEKS (3).

LEAR - LOW MORTALITY, EQUAL TO CONTROL (2).

1. Beare *et al*., J. Nutr. 80, 157 (1963).
2. Beare-Rogers and Nera, Lipids 9, 365 (1974).
3. Hulan *et al*. Lipids 11, 6 (1976).

HEART MITOCHONDRIA OF RATS

FED HEAR

1970 - impaired respiratory activity (1)
- partial loss of respiratory control (1)

1973 - properly isolated mitochondria are coupled and fully active (2)
Confirmed (3 - 6)
- Uncoupling due to artifacts (free fatty acids)(3).

FED LEAR

1973 - Coupled and fully active (2)

1. Houtsmuller et al., BBA 218, 564 (1970).
2. Kramer et al., J. Nutr. 103, 1696 (1973).
3. Dow-Walsh et al., BBA 396, 125 (1975).
4. Cheng and Pande, Lipids 10, 335 (1975).
5. Beare-Rogers and Gordon, Lipids 11, 287 (1976).
6. Galli et al., Lipids 11, 670 (1976).

MYOCARDIAL LIPIDOSIS - HEAR

SPECIES	% 22:1	SEX	SEVERITY	MAXIMUM
Rat	22-48	M, F	severe	3-7 days
Duckling	37-50	M, F	severe	continuous
Chick	22, 32	M	severe	3-14 days
Pig	22-45	M, F	mild	1-4 wks
Monkey	25, 29	M, F	mild	2-8 wks

<u>SUMMARY</u>: All animals tested showed lipidosis
Extent and response differs between species
No sex difference

MYOCARDIAL LIPIDOSIS - LEAR

SPECIES	% 22:1	SEX	SEVERITY	MAXIMUM
Rat 0.1	0.1-4	M, F	none-mild	1 wk
Duckling	12-25	M, F	none-mild	continuous
Chick	1-4	M	none-mild	3-14 days
Pig	4, 15	M, F	none-mild	1-4 wks
Monkey	0.2	M, F	none	2-8 wks

SUMMARY:

Lipidosis none or very mild in all species

Directly related to 22:1

22:1n-9 (erucic) > 22:1n-11 (cetoleic)

22:1n-9 > 20:1n-9

Diet (% 22:1)	Time on Diet (days)	TG (µg/g)	FFA (µg/g)	Ref.
Control	0	6,200	4,900	1
RSO (45%)	3	62,100	4,500	
Control-18:1 (72%)	7	3,800	2,900	2,3
Synthetic oil (73%)	7	47,500	8,300	
Corn	3	3,700	1,600	4
RSO (22.3%)	3	19,700	2,600	

1. Houtsmuller _et_ _al_. BBA _218_, 561 (1970)
2. Beare-Rogers _et_ _al_. Lipids _7_, 46 (1972)
3. Beare-Rogers _et_ _al_. Lipids _7_, 548 (1972)
4. Dow-Walsh _et_ _al_. BBA _396_, 125 (1975)

Cardiac free fatty acid levels (µg/g) in male rats fed HEAR oil

Method	Time on Diet (days)		
	0	3	7
Standard method	100	1,800	1,700
Improved method	40	210	210

Kramer and Hulan, JLR _19_, 103 (1978)

Diet (% 22:1)	Time on Diet (days)	TG (µg/g)	FFA (µg/g)
Soybean oil	3	2,000	95
	7	2,000	113
	112	1,700	47
LEAR (0.2%)	3	1,400	73
	7	3,200	98
	112	1,500	46
HEAR (22.3%)	3	14,400	210
	7	29,300	210
	112	4,500	68

Kramer and Hulan unpublished data

Cynomologus monkeys fed soybean oil or Tower RSO for up to 24 weeks

Diet	Sex	Affected/Examined
Soybean oil	Male	2/7
	Female	0/7
Tower RSO	Male	0/14
	Female	0/12

Kramer _et al_. Can. J. Anim. Sci. (in press)

Comparative studies

Monkeys - male and female
Pigs - boars and gilts
Rats - male and female
- albino and hooded

Summary of Agriculture Canada data on male rats fed diets containing 20% by weight fat or oil in the diet for 16 weeks

Diet (% 22:1)	Affected/Examined	%	No of expts.
Lard	14/75	19%	2
Corn oil	17/233	30%	7
Olive oil	38/120	32%	2
Safflower oil	7/20	35%	1
Soybean oil	84/181	46%	3
Span RSO (4.5%)	103/159	65%	7
Zephyr RSO (0.9%)	117/161	73%	6
Tower RSO (0.2%)	154/220	70%	6

MYOCARDIAL LESIONS - PIGS

Species	RSO % 22:1	No. Sex	Time on Diet (wks)	Affected/Examined				Ref.
				Control	(%)	RSO	(%)	
Yorkshire	4	96M,96F	1-16	34/120	(28)	22/72	(31)	1
Crossbred	4,21	56M,56F	16	no difference				2
Yorkshire	22	72M	16	2/48	(4)	3/24	(13)	3
Norwegian Landrace	44	40 (M&F)	1-52	no difference		10/40	(25)	4
Crossbred	0.3, 1.2 4.9, 34	40M,40F	4-23	3/16	(19)	2/62	(3)	5
Yorkshire	1,24	60M	24	20/30	(67)	22/30	(73)	6

1. Friend et al. Can. J. Anim. Sci. 55, 49 (1975).
2. Aherne et al. Can. J. Anim. Sci. 55, 77 (1975).
3. Friend et al. Can. J. Anim. Sci. 55, 571 (1975).
4. Svaar and Langmark, Action Thematique, INSERM, Paris, 329 (1975).
5. Aherne et al. Can. J. Anim. Sci. 56, 275 (1976).
6. Friend et al. Can. J. Anim. Sci. 56, 361 (1976).

Effect of sex on myocardial lesions in rats

Sex	5% Corn oil	20% Corn oil	20% Zephyr RSO
Male	3/26	7/26	16/25
Female	0/26	4/26	3/26

χ^2 Analysis

	χ^2	d.f.
Male vs. Female	14.93 ($P < 0.001$)	1

Hulan *et al*. Can. J. Physiol. Pharmacol. 55, 265 (1977)

Effect of castrating on myocardial lesions

Animals	5% Corn oil	20% Corn oil	20% Zephyr RSO
Entire male	3/26	7/26	16/25
Castrated male	1/26	1/26	9/27

χ^2 Analysis

	χ^2	d.f.
Entire vs. Castrated male	9.91 ($P < 0.001$)	1

Hulan *et al*. Can. J. Physiol, Pharmacol. 55, 265 (1977)

Effect of dietary fat levels on myocardial lesions

Experiment	5% Corn oil	20% Corn oil
A	6/101	23/99
B	4/104	13/104

χ^2 Analysis

	χ^2	d.f.
A: 5% vs. 20% Corn oil	15.04 ($P < 0.001$)	1
B: 5% vs. 20% Corn oil	5.78 ($P < 0.05$)	1

Hulan *et al*. Can. J. Physiol. Pharmacol. 55, 258 (A) & 265 (B) (1977).

MYOCARDIAL LESIONS - RATS - EFFECT OF STRAIN

Strain of rat	Corn oil 5%	Corn oil 20%	LEAR oil 20%
Sprague-Dawley-C	8	23	88
Sprague-Dawley-A	4	17	45
Wistar	12	17	64
Sherman	0	36	71
Chester Beatty	0	20	0

Hulan _et al_. Can. J. Physiol. Pharmacol. _55_, April (1977).

Myocardial lesions - differences between albino and hooded rats

Strain	Corn oil	Zephyr RSO	HEAR
Sprague-Dawley (albino)	8/24	20/24	24/24
Chester Beatty (hooded)	4/24	6/24	4/23

Kramer, Hulan, Trenholm and Corner (unpublished data).

Cause of myocardial lesions in male rats

1. Long-chain monoenes (20:1, 22:1, 24:1)
2. Toxic compound in rapeseed oils
3. Fatty acid imbalance

Is 22:1 the cause of heart lesions in rats?

Evidence for:

Diet	% 22:1		Lesions
LEAR oils still contain some 22:1	0.2-5%	>	control
Sunflower plus trierucin (1:1)	42%	>	control
Erucic acid esterified into olive oil	30%	>	control

Is 22:1 the cause of heart lesions in male rats?

Evidence against:

Diet	22:1		Lesions
22:1 present at low levels			
Tower RSO	0.2%	>	control
22:1 added without effect			
Lard plus 22:1	5.6%	=	lard
Soybean oil plus 22:1	5.7%	=	soybean
Tower RSO plus 22:1	0.8%	=	Tower RSO
Tower RSO plus 22:1	5.9%	=	Tower RSO
22:1 esterified without effect			
Rendered pig fat	5.6%	=	control
Erucic acid esterified with olive oil	3%	=	olive oil
Simulated HEAR (no 18:3)	28.7%	=	soybean oil
Canbra (2.9%, 22:1) hydrog. IV78	2.6%	<	canbra
Mixing Brassica with non-Brassica oils			
HEAR and lard/corn oil (10:10)	19%	=	control
HEAR and sunflower oil (15:25)	16.5%	=	control

Myocardial Lesions in Rats fed Tower RSO or its Fractions

Fractions	% Sterol	Incidence
Soy	0.10	22/46
Soy - MD7	0.01	25/46
Tower RSO	0.51	35/46
Tower RSO - MD7	0.02	33/46
Tower RSO - AC-F2A	0.001	19/46
Tower RSO - AC-F2B	0.009	29/46

Unpublished data

Stepwise multiple regression analysis

			% variation explained
Step	1	Experimental variation (EV)	29.3
	2	EV & 16:0	69.4
	3	EV & 16:0 & 18.3	75.2
Final Step		EV & all dietary fatty acids	76.6
Step	2	EV & 18:0	58.8
	3	EV & 18:0 & 18:3	67.7

MYOCARDIAL LESIONS IN RATS FED SPAN RAPESEED OIL OR ITS FRACTIONS

Fractions	% Sterol	Incidence	
Span	0.25	23/50	N.S.
Molecular distillate - 5	0.01	31/50	
- 6	0.03	24/50	
- 7	0.003	40/50	

Kramer *et al.*, Lipids *10*, 505 (1975) and *10*, 511 (1975).

MYOCARDIAL LESIONS IN RATS FED SPAN RAPESEED OIL OR ITS FRACTIONS

Fractions	% Sterol	Incidence	
Span	0.25	6/10	N.S.
Ads. Chrom. - F2	0	6/10	
Span	0.25	17/20	
Ads. Chrom. - F1	0.64	19/20	N.S.
-F2	0	16/20	
- F3	0.006	16/20	

Kramer et al., Lipids 10, 505 (1975) and 10, 511 (1975).

Legend to Figures

Figure 1. Observed vs. predicted incidence of myocardial lesions based on the regression analysis. Results are from 3 laboratories: ■ Agriculture Canada, ▲ Health and Welfare Canada, O Unilever Research Laboratories, The Netherlands.

Figure 2. Data in Figure 1 plotted according to kind of test oil or fat: ▲ LEAR oils, ■ control oils or fats, O mixtures of LEAR and control oils and fats.

Figure 1

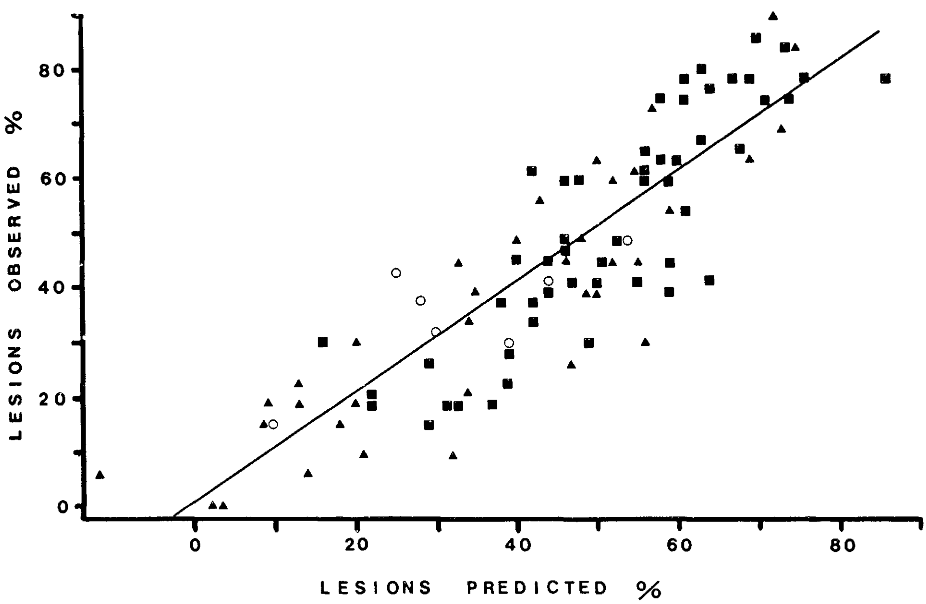
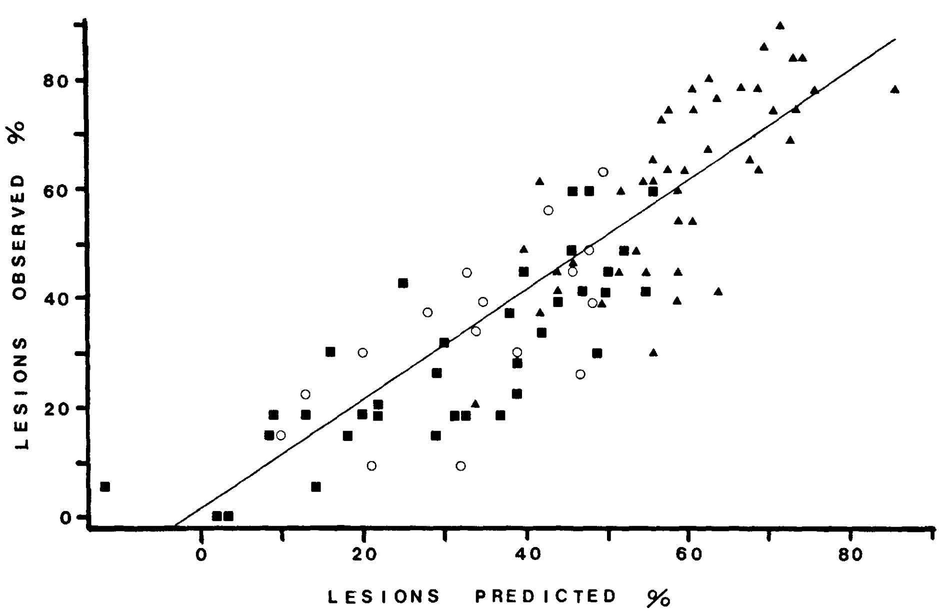


Figure 2



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SYMPOSIUM ON
NEW VARIETIES OF COLZA
HELD IN BRUSSELS ON
11 April 1978

Information presented by Mr J. FLANZY
National Livestock Research Centre
Nutrition Research Station
National Agricultural Research Institute (INRA)
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Comparative physio-pathological effects of ingestion of groundnut oil and Primor rapeseed oil
1974 - 76 INRA study programme

In 1974 examination of the work carried out on new rapeseed oils with a low erucic acid content showed that different results were obtained by the various laboratories. Some laboratories found that these oils still caused myocardial lesions, less severe than those obtained with oils rich in erucic acid but more pronounced than those observec in the case of the control oils. Other laboratories found that the effects of this new oil were no different from those of the other food oils tested in that where lesions existed they were equally severe irrespective of the oil used. This disagreement between researchers could possibly be explained by the choice of experimental procedure, i.e. use of different oil dose, animal species and techniques by the various laboratories.

In 1974, therefore, the INRA decided to launch a research project along new lines : the main part of the work was based on histological analyses of the myocardium of rats; teams of French and foreign researchers, well-known for their research into rapeseed oils, cooperated on the project; the same material was examined by all these histologists, a precaution which ensured that the experimental procedure used did not give rise to any variations; lastly, the histological analysed were carried out "blind" twice.

Parallel to this work an electrophysiological study of the myocardium of rats and an experiment on laying hens were also carried out.

Tasks were allocated as follows :

- Preparation of the experimental procedure, breeding of animals, measurement of their growth and the weight of their organs - G. Rocquelin, INRA, Dijon.
- Preparation of slides. Histological examinations of organs other than the heart - X. Fouillet, IFFA - CREDO[1].
- General anatomical examination of the myocardium - O. Schmitt, INRA, Jouy-en-Josas
- Ultrastructural study of the myocardium - P. Y. Hatt, Léon-Bernard Hospital (Limeil-Brevannes); N. Vodovar, INRA, Jouy-en-Josas.
- Electrophysiological study of the myocardium - E. Coroboeuf, Paris University, XI (Orsay).
- Research on laying hens - B. Leclerq, INRA (Nouzilly).
- Director of the study programme - J. Flanzy, INRA, Jouy-en-Josas.

The participants unanimously agreed on the following summary of the main results of this project.

1. In rats, after a diet of six months, the nature of the oil had no effect on the body weight and the weight of the myocardium.
   The relative weight of the liver and kidneys was slightly higher in the Primor batch than in the groundnut batch.
   After a one-year diet the weight of the rats in the Primor batch was 7 % lower than that of the animals in the groundnut batch. However, the relative weight of the heart and liver in the Primor batch was higher than in the groundnut batch.

1 Centre de Recherche et d'Elevage des Oncins (IFFA-CREDO) Saint-Germain sur l'Arbreste - 69210 - France.

2. The anatomopathological study of the liver, lungs, spleen and kidneys did not reveal any difference between the batches of rats on a one-year diet containing either 5 % Primor or 5 % groundnut oil.

3. The general anatomical examination of the myocardium of rats did not indicate that the dose (5 - 10 - 15 % of the ration) or the oil (Primor - groundnut) had any effect. The duration of the diet (6 to 12 months) increases the number of lesions.

4. The optical microscopy results demonstrated that a diet containing 5 or 10 % oil did not reveal any significant difference after a six-month experiment according to whether Primor rapeseed oil or groundnut oil was used. Some researchers concluded that "the cardiopathogenic effect of the diet containing 15 % Primor oil is greater than that of the diet containing groundnut oil"; according to others," the diet containing 15 % Primor oil is more fibrogenic than that with a 15 % groundnut oil content. When the histiocytic lesions are taken into account the differences lose their significance." The results obtained after a one-year diet containing 5 % oil showed an increase in the number of rats with lesions but it was not possible to distinguish between the effects of the Primor rapeseed oil and those of groundnut oil.

5. Electronic microscopy revealed variations in the size of the mitochondria (giant mitochondria). Some researchers held that the changes in the myocardium were much greater in rats fed Primor rapeseed oil, while others saw no obvious difference between the two oils. However, it was generally agreed that the cardiotoxicity of oils increased in proportion to the ration content and the duration of the diet.

6. No significant difference was noted between the cardiac cell electrical phenomena of animals fed either rapeseed oil rich in erucic acid or groundnut oil for 15 days.

7. In the cas of laying hens rapeseed oil with a low erucic acid content did not have any effect on the weight of the egg and the yolk nor on the laying rate, unlike rapeseed oil rich in erucic acid. Embryonic mortality was generally lower where groundnut oil was used than where Primor or soya oils were used.

Symposium on "New varieties of colza"

Physiological effects of low erucic-acid colza oils in the diet

Prof. Dr. A. Seher
"Bundesanstalt für Fettforschung"
D-4400 Münster

Brussels, 11 April 1979

Summary

At the present stage it is impossible to say which fat is the most suitable for human consumption. There is no doubt that a persons's fat requirements vary in type and quantity according to his age and occupation. But man is not a laboratory animal. His diet is varied and comprises ever-changing combinations of fats, contained either noticeably or otherwise in a variety of foods. With regard to the consumption of colza oil low in erucic acid, it may be confidently stated that according to current scientific knowledge such oil presents no nutritional hazards.

Erucic acid has ceased to present problems since the development of new varieties of colza with seeds containing distinctly less than 1 % erucic acid. Farmers in Federal Germany all use these new varieties nowadays. The continued improvement of seed has largely removed the initial agricultural problems. The linolenic acid content of the new high-quality colza oils is higher than that of the old oils. Any scientific questions still unanswered are on the whole not specific to colza oil but apply to all edible oils containing linolenic acid. However, in view of prevailing consumption patterns in our society, no nutritional risks are to be feared as a result of consumption of the new low-erucic-acid colza oil.

Symposium on new varieties of colza

Physiological effects of low erucic acid colza oils in the diet

Prof. A. Seher
(Bundesanstalt für Fettforschung)
D-4400 Münster

Brussels, 11 April 1978

1. Introduction

It is generally known that the earlier types of colza oils with their high erucic acid content caused severe damage to rats and many other laboratory animals. To answer Mr. Schiratti's question as to how high erucic acid colza oil came to be exported to non-Member countries, it must be pointed out that, so far, no harm to human health has been traced back to colza oil either in Europe, where fat consumption is high but colza oil consumption limited, or in those regions of India where colza oil is practically the only oil in a low fat diet.

As a reaction to the warnings which followed animal experiments, only newly developed, low erucic acid colza varieties have been grown for consumption in the Federal Republic of Germany since 1974. Consequently, all colza oil produced since summer 1975 for human consumption has contained no more than 5-7% erucic acid. The drop in yield resulting from the change to new varieties, which caused farmers problems at first, has now been largely made up.

When this conversion to new varieties began, the research findings - mentioned several times today - of the Roquelin team in France and Mrs. Baere-Rogers in Canada already indicated that even low erucic acid colza oils cause damage to cardiac muscle in test animals. This symposium bears witness to the fact that many laboratories throughout the world then began to investigate the nutritional and biochemical effects of low erucic acid colza oils. In the Federal Republic of Germany, the "Bundesanstalt für Fettforschung" has been working in this field since the changeover to new varieties in 1974 and has devoted considerable resources to the research.

As the first slide shows, a low erucic acid colza oil differs from other edible oils in the following ways:

Slide 1

1. by its residual content of 0.5-5% erucic acid
2. by its content of oil-soluble cleavage products from the glucosinolates
3. by its very high level of oleic acid and linoleic acid/linolenic acid ratio of about 2:1.

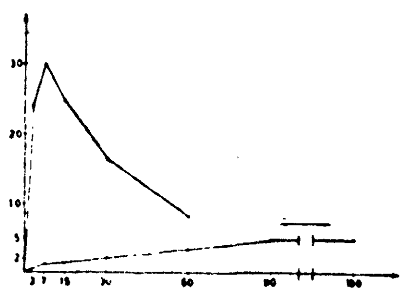
We are currently investigating the nutritional implications of these characteristics of colza oil. I would like to report on the progress to date, dealing first with the tests conducted on rats and then with those on pigs will be commented on more fully because the pig metabolism is closer to human metabolism.

2. Effects on the heart

The next slide shows the presence of erucic acid in the cardiac muscle of rats and pigs.

If rats are fed with high erucic acid colza oil, there is a build-up of non-degradable erucic acid in cardiac muscle, causing lipidosis in proportion to the dose administered.

Slide 2



As the upper curve shows, the maximum build-up of erucic acid occurs after about 7-10 days in the rat. The fat content of the cardiac muscle cells increases more than tenfold. Then, however, the fat content and the erucic acid concentration slowly sink again.

The lower curve shows that the situation is different in the pig. Lipidosis scarcely ever occurs but there is a slow build-up of erucic acid in the lipids of the cardiac muscle cells.

Fat metabolism takes place in the mitochondria of the cells. In our efforts to explain the differences, we examined the behaviour of isolated mitochondria from rat and pig hearts.

The table shows the relative rates of oxidation of several fatty acids after 30 minutes' incubation with heart mitochondria. For easier comparison of the conversion rates, the quantity of oleic acid transformed in 30 minutes has been taken as 100%.

Slide 3

Table 4

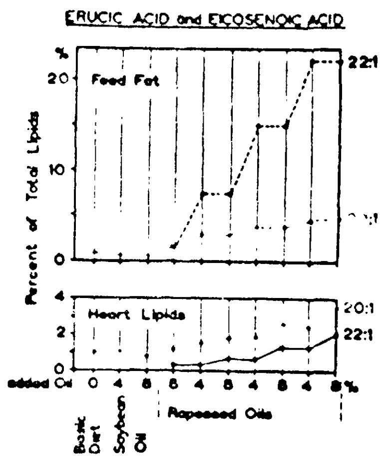
Relative oxidation rates of 1-^{14}C labelled fatty acids after 30 min incubation time

Fatty acid (100 m mol/mg protein)	Rat heart mitochondria	Pig heart mitochondria
Oleic acid	100%	100%
Palmitic acid	88%	94%
Erucic acid	1.8%	5.7%
Nervonic acid	0.5%	-

In the rat heart mitochondria, the quantity of erucic acid oxidized in 30 minutes is only 1.8% of the quantity of oleic acid oxidized. The corresponding rate is 5.7% in pig heart mitochondria. Although absolute metabolic activity in rat hearts is far higher than in the pig, the pig heart can cope with three times more erucic acid than that of the rat.

On the other hand, the pig heart is clearly incapable of the adaption process observed in rats. As the administration of erucic acid in the diet continues, there is no drop in the erucic acid content of the heart lipids of the pig.

Slide 4



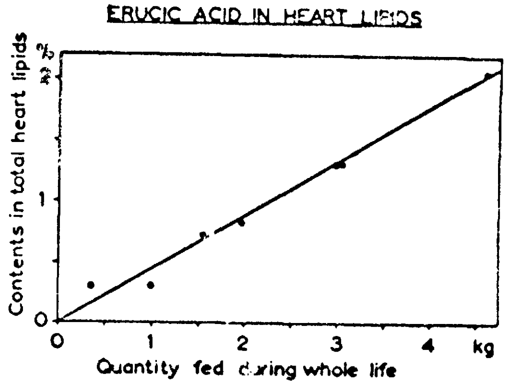
After a feeding period of 100 days, the quantity of erucic acid in the total lipids of the heart muscle of pigs corresponds to the quantity administered in the diet.

The upper part of slide 4 shows the concentration of erucic acid in the fats fed to 10 feeding groups. The first three groups received no colza oil, while the seven others received four different concentrations of erucic acid ranging from 1.7% to 22.5%. The highest concentrations were administered in rations supplemented with 4% and 8% fat respectively. The basic feed consisted of barley and soya cake, with added mineral salts, trace elements and vitamins.

The lower part of the slide shows the erucic acid contents found in the cardiac muscle of each feeding group. It is to be noted that even the lowest concentration (1.7%) of erucic acid gives rise to an erucic acid content in the heart lipids.

Under these feeding conditions, the erucic acid content in cardiac muscle increases stepwise. However, the stepwise progression of the lower part of the graph is different from that of the upper part. This drew our notice to another relationship.

Slide 5



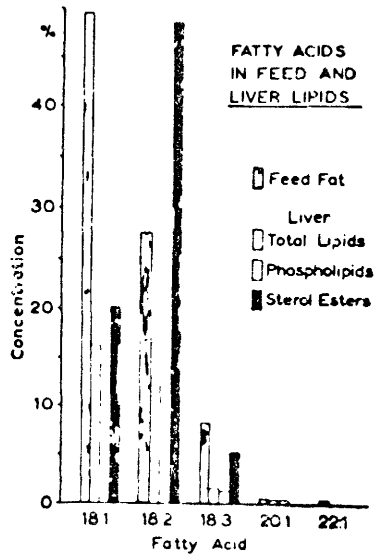
In slide 5 the abscissa shows the amount in kg of erucic acid fed to the animals throughout their lives. The ordinate shows the percentage erucic acid content in the total cardiac lipids of the same animals. There is a high correlation.

The curve shows quite clearly that erucic acid does not have a no-effect level, at least where pig hearts are concerned. Even when 340 g of erucic acid are administered over 100 days in 200 kg of feed, small quantities of erucic acid are deposited in the cardiac lipids. This shows how important it was to develop low erucic acid varieties of colza.

It is impossible to say, however, whether this small amount of erucic acid would have had any significant physiological effect if the animals had been allowed to grow old. Nor can it yet be said whether, or how rapidly, erucic acid disappears from cardiac muscle lipids if the feedingstuff containing colza oil is withdrawn.

3. Effects on the liver

The mitochondria of pig liver have the capacity to eliminate erucic acid rapidly.

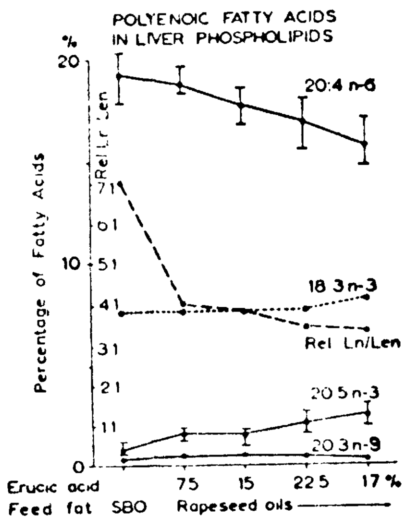


The diagram shows the levels of the principal fatty acids in the feed in the total liver lipids, the metabolically active phospholipids and the sterol esters. The data were gathered from the group of animals which had received a ration containing 8% colza oil in which the erucic acid concentration was only 1.7% for 100 days.

The oleic acid, which predominated in the feed, was reduced to 20% or less in all lipid fractions. The erucic acid could not be detected in any of the hepatic lipids. It is decomposed to oleic acid via eicosanoic acid and clearly follows the liver's metabolic breakdown route for oleic acid.

The essential linoleic acid is markedly enriched in the sterol esters. These esters also contain more linolenic acid than the phospholipids and the total lipids. From the linoleic acid in the organism is formed the arachidonic acid which is particularly important for the liver functions and which is not present in the feedingstuffs.

Slide 7



In the liver, the carbon chain of the linoleic acid is lengthened by two atoms. In addition, two new double bonds are formed so that the fatty acid with 18 carbon atoms and two double bonds is transformed into arachidonic acid with 20 carbon atoms and four double bonds, the last one being six carbon atoms removed from the final methyl group. In slide 7, the upper curve shows the arachidonic acid content in the liver phospholipids of the animal groups whose feed contained 8% added fat. In the first group (which received soya oil) the highest level of arachidonic acid was found. In the case of animals fed with colza oil, the arachidonic acid content shows a continuous fall. This fall is clearly unconnected with the erucic acid content of the feed (bottom of the graph), which although it at first increases is lowest in the last group. Another component of the fats must be responsible for this reduction of arachidonic acid in the liver.

In this series, the linoleic acid content of the fats fell from 50 to 26%. The reason for the drop in arachidonic acid formation could be a deficiency of linoleic acid. It is known that a linoleic acid deficiency in the body gives rise to the formation of increasing quantities of eicosatrienic acid. The lower curve shows that the level of this acid remains constantly low, so there can be no deficiency of linoleic acid and the drop in arachidonic acid formation must have another cause.

I would now like to draw your attention to another pair of curves. The dotted curve in the middle represents the slow increase of linolenic acid in the diet. The parallel curve represents the increase of eicosapentaenic acid which is formed in the liver from linolenic acid. In both acids the last double bond is three carbon atoms removed from the final methyl group. In chemistry, we speak of the n-3 family. In the rat, this acid is converted largely into hexaenic acid (22 : 6n-3) but this was not noticed in the pig. This finding may have a bearing on the differing histological observations in the rat and the pig; this aspect merits further investigation.

The interaction of the various polyenic acids in the body is not yet thoroughly researched. However, it may be presumed that the quantitative relationship between linoleic acid and linolenic acid plays some role. The broken line in the middle of the slide shows that in our feeding tests this ratio tends towards ever-lower values. It is possible that the saturated fatty acids also play a part in this process.

In 1963 Mohrhauer and Holman noted that an increased quantity of linolenic acid in rat feed led to a lower level of arachidonic acid in the body. More recently, Lemarchal and his team in France and Baere-Rogers and Nera in Canada have carried out similar experiments, especially with rats, and have reached similar conclusions.

The reduced arachidonic acid synthesis in the liver affects all the organs of the experimental animals.

The interaction between linoleic acid and linolenic acid in metabolism is not a problem specific to colza oil; it concerns all edible fats containing linolenic acid. As regards human nutrition, it must be clearly understood that man's diet is never as monotonous as the diet of laboratory animals and that similar effects are therefore very unlikely in man.

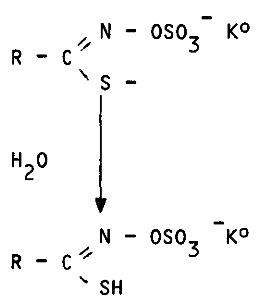
4. Histopathological examinations

In earlier tests on the effect of erucic acid, morphological changes in the cardiac muscle of rats and many other animals were observed. From pigs fed by us, Dr. Vles and Mr. Bijster took, according to statistical rules, nearly 3 000 sections of cardiac muscle and examined them under the microscope. In none of the feeding groups, including that with 22.5% erucic acid in the fat fed, were there any significant differences from the control groups. It should be mentioned that the lesions of individual cells or of cell groups in cardiac muscle, which are described so often and so exactly in erucic acid research, are not specific to erucic acid but represent a general reaction of cardiac muscle to nutritional or other stress.

Fat research has long been neglected compared with carbohydrate and protein research and, unfortunately, the present tests have been the first to show how these changes in cardiac muscle should be evaluated. The uncertainty about low erucic acid colza oils which existed a few years ago has now been dispelled.

In conclusion, mention should be made of a group of components of colza oil which distinguish Cruciferae oils fundamentally from all other edible oils. These are the breakdown products from the glucosinolates. In most plants of the Cruciferae family, to which cabbages belong, and in some quite different sorts of plant, e.g. spurges, there occur compounds of sulphur and nitrogen with glucose, called glucosinolates. The basic chemical formula is shown in the next slide.

Slide 8



The molecule contains sulphur, nitrogen and glucose. There are about 70 known glucosinolates which differ from one another only in the nature of the residue "R". In colza plants there are six to eight of these compounds. The enzyme myrosinase, which occurs only in plants, cleaves these compounds into glucose and aglucone. The aglucone is not stable and decomposes according to the scheme in the next slide.

Slide 9

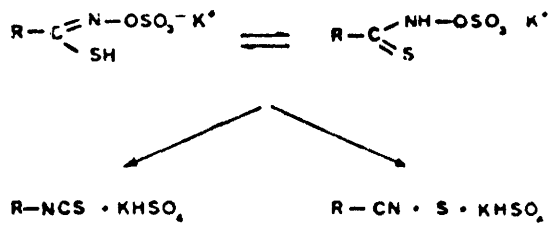


Fig. 9.

It decomposes mainly to give mustard oils, which are named after the condiment in culinary mustard. Nitriles are also formed in smaller amounts.

Only a very small proportion of these substances is transferred to the oil during the oil-manufacturing process. The majority remains in the residues. Most of the compounds contained in the raw oil are removed by refining.

In order to detect any possible unfavourable effects of these compounds or of other substances not yet identified (Baere-Rogers x factor), Mangold and his team isolated fractionated extracts of colza seed and fed them to test animals.

Slide 10

Colza seed : Lesira (a) Rapol (b)	Hexane Extraction	→	Surface lipids Fraction I.a I.b
Grinding			
Colza meal without surface lipids	70% aqu Acetone Extraction	→	Glucosinolates Fraction II.a II.b
Colza meal without surface lipids and without glucosinolates	Hexane Extraction	→	Oil Fraction III.a III.b

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Colza seed : Lesira (a) Rapol (b)			
Grinding			
Colza meal untreated	Hexane Extraction	→	Oil Saponification, Extraction Unsaponifiable Material Fraction IV.a IV.b

To begin with, the authors isolated the surface lipids (Fraction I), by hexane extraction, from intact seeds of the low erucic acid variety "Lesira" and the high erucic acid variety "Rapol". Then the seeds were ground and the glucosinolates extracted with aqueous acetone (Fraction II). The residue was then defatted, giving an oil free from the substances normally contained in colza (Fraction III). At the same time, other colza seed was subjected to the usual extraction process and the unsaponifiable matter (Fraction IV) was isolated from the oil after saponification.

Each of these fractions was dissolved in soya oil and fed to rats. Even though the quantities of surface lipids and Fraction IV had been increased fivefold, none of the test animals suffered any harmful effects. No organ changes nor deviations from the control group, which had received pure soya oil in its feed, were detected microscopically, except in the case of rats which had received oil with a high erucic acid content (Fraction III.b). Similar findings were made by Mr. Kramer, who described them in his report this morning.

ANTI-THROMOGENIC EFFECT OF RAPESEED OIL PRIMOR IN RAT. APPLICATION TO MAN.

S. Renaud and L. McGregor
INSERM, Unit 63, Lyon-Bron, France

Summary

Utilizing our experimental model of thrombosis initiated by a bacterial endotoxin, rats (12 to 24 per group) have been fed for 11 weeks, hyper-lipemic diets containing in weight 10% butter and 24% of one of the rats mentioned hereafter. The results indicate that the fats studied could be classified according to their thrombogenic properties, in the following decreasing order.

1 - Saturated margarine. 2 - Palm oil. 3 - Butter. 4 - Rapeseed oil, erucid acid rich. 5 - Hydrogenated coconut oil. 6 - Corn oil. 7 - Rapeseed oil Primor. 8 - Tournesol margarine.

Fats No 6, 7 and 8 were also associated with the lowest plasma chlores-terol.

In farmers from Moselle, a region with a high incidence of coronary heart disease and high intake of saturated fats, 55 families have accepted to modify their dietary habits while an equal group serve as controls.

The fats exclusively utilized for cooking or other purposes in the families having changed their dietary habits were margarine or oil con-taining from 50 to 100% of Primor oil. The mipemia, blood caogulation, the platelet composition and function (the factor the most clearly associated to the thrombotic tendency and probably to coronary heart disease), as well as the electrocardiogram, have been determined before, and one year after modification of the dietary habits. The taste and other properties of the margarine and oil containing Primor, have been well accepted by the majority of the families. The results after one year will be reported on 25 families

ANTI-THROMBOGENIC EFFECTS OF PRIMOR-RAPESEED OIL IN RAT - APPLICATION TO MAN

S. Renaud and L. McGregor

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EXPOSE

Since most of the audience does not appear to be essentially medical, it might be useful to briefly describe what are the cardiovascular diseases which we are going to talk about or which have already been mentioned before describing the role of rapeseed oil in these problems.

The erucic acid rich rapeseed oil certainly induces in various animal species cardiac lesions, called myocardiopathies, but this type of lesion in man is extremely rare in comparison to coronary heart disease which is responsible, in industrialized countries, for 30 to 50% of the death rate, particularly in human males.

The coronary heart disease or CHD presents mostly 3 different clinical manifestations:

1. angina pectoris, a constricting distressing pain within the chest occurring during physical exercise (walking), disappearing almost immediately at rest.
2. heart attack or myocardial infarction resulting from the total obstruction of a coronary artery, the vessels feeding the heart.
3. sudden death, dramatic form of the heart attack which might affect 30% of all the cardiac subjects, even before any other manifestation.

Coronary heart disease starts in the first decades of life, by lipid accumulation within the wall of arteries (atherosclerosis) and progresses until the lumen of the vessels and the amount of blood passing in the arteries are considerably reduced. When the reduction in the inside diameter is of approximately 70%, angina pectoris might occur. If a thrombus (coronary thrombosis) in other terms an occluding plug, essentially composed of blood platelets and fibrin, the elements of a blood clot, is superimposed over an atherosclerotic plaque, this event totally blocking the supply of blood to a part of the heart, an infarct results which means the death of the cells located in the affected area (figure 1). This obstruction might induce a fibrillation, the heart does not pump blood effectively, resulting in a sudden death.

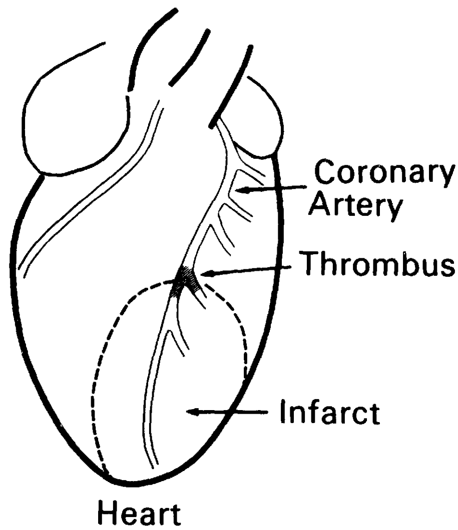


Fig. 1: Schema of a cardiac infarct resulting from the complete obstruction of a coronary artery by a thrombus.

Other less common problems are also associated with atherosclerosis and thrombosis. They are

1. Peripheral arteriopathy responsible for intermittent claudication (phenomenon similar to angina pectoris but in the legs) and eventually for gangrene.

2. Brain thrombosis (or hemorrhage) which result in various form of paralyses and even death.

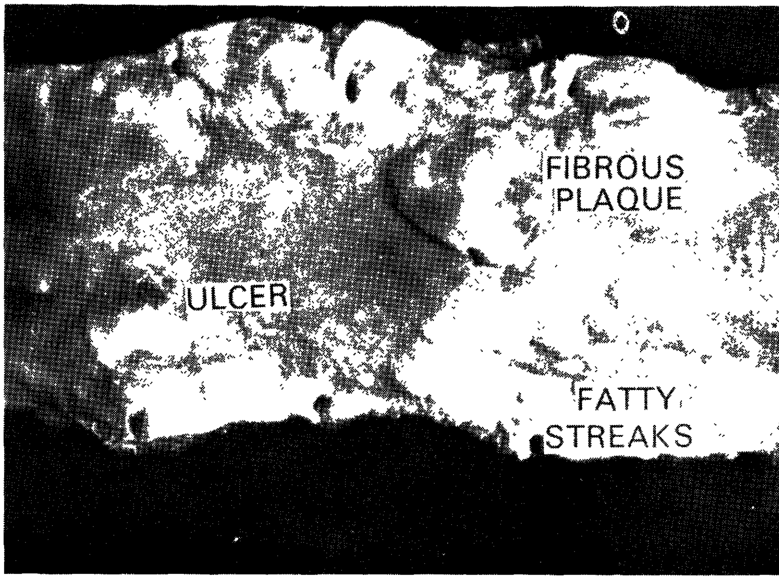


Fig. 2: A human artery (aorta) opened longitudinally and presenting the main atherosclerotic lesions (fatty streaks, fibrous plaque, ulceration).

In figure 2 is shown a human artery (aorta) opened longitudinally and presenting the three main type of atherosclerotic lesions which are the fatty streaks (the earliest lesion), the fibrous plaque (atheroma) and the ulceration, the ultimate stage in the disease.

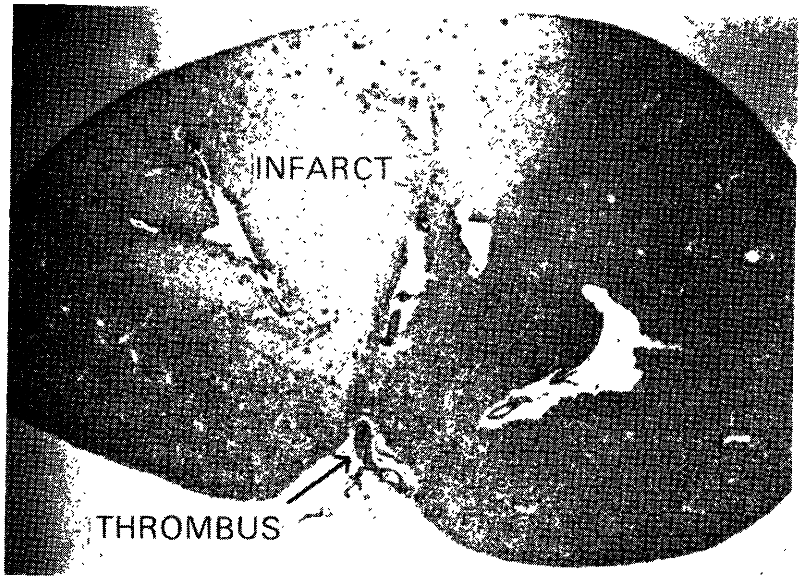


Fig. 3: Histologic slide of an infected kidney (renal infarct). Note the triangular form of the infarct resulting from the occlusion by a thrombus (Thr) of a renal artery.

Figure 3 is a photograph of an histologic slide, showing an infarcted kidney in rat, with its typical geometric form and the dead part in the distribution territory of the thrombosed artery.

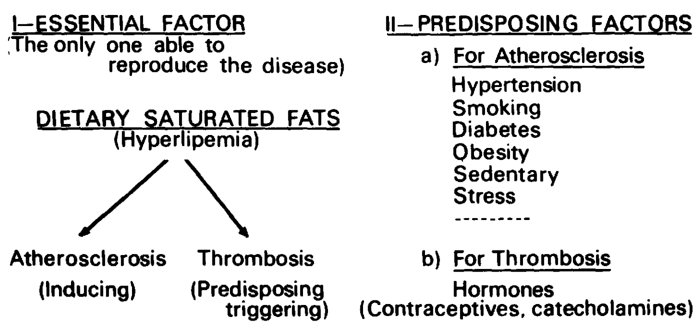


Fig. 4: Risk factors for coronary heart disease.

What are the responsible factors for coronary heart disease and consdquently for atherosclerosis and thrombosis. In figure 4 are listed the various risk factors for CHD, in other terms, the various factors which have been shown in numerous studies, to be associated with the disease. They are hypertension, diabetes, cigarette smoking ... One of these factors appear to have a key role; we have called it the essential predisposing factor for CHD. In the absence of this factor CHD apparently never occurs, while when present, even alone, this factor induces the disease. This factor is the consumption of saturated fats. We have the evidence that CHD is associated with the saturated fat intake since 1930, all the results since then having confirmed the earlier results, in particular those of A. Keys which have shown that this factor is the environmental factor the most closely associated with the disease (Keys A., Coronary heart disease in Seven Countries. Circulation 41: suppl. 1, 1970).

As already indicated CHD means atherosclerosis and thrombosis. Dietary fats appear to have an effect on both of these phenomena. Let us examine first their effect on atherosclerosis.

From a chemical point of view, atherosclerosis is characterized essentially by accumulation of certain lipids, mostly cholesterol esters (figure 5) in the arterial wall while fibrous tissue, represented here by collagen as compared to the total weight of the artery, presents solely minor modifications with the age or the severity of the lesions.

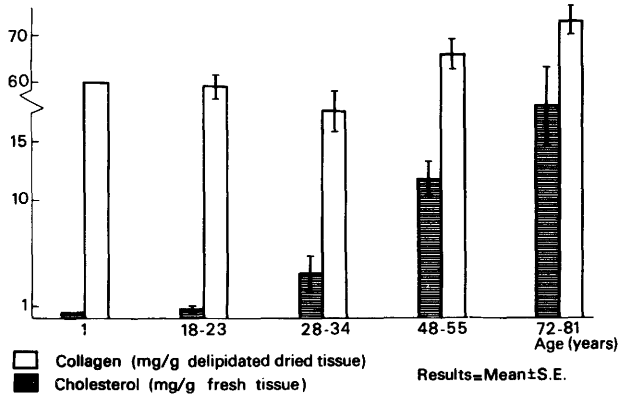


Fig. 5: Total cholesterol and collagen content of the aorta at different ages in French males from Lyon (accidental death)

This type of lesion can be almost perfectly reproduced in most of the animal species provided that serum cholesterol can be successfully increased. For example in rabbit, by adding to the diet 10 to 20% of saturated fat, for example butter, one can reproduce as illustrated in figure 6, the curves of cholesterol and collagen accumulation with time in the aorta, similar to this observed in man. A similar result, at least to our knowledge, cannot be reproduced either by replacing saturated by polyunsaturated fats, or by increasing the level of proteins or of carbohydrates. Only the saturated fats appear to conduce to the results observed here.

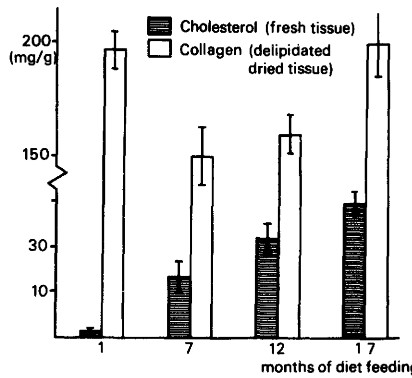
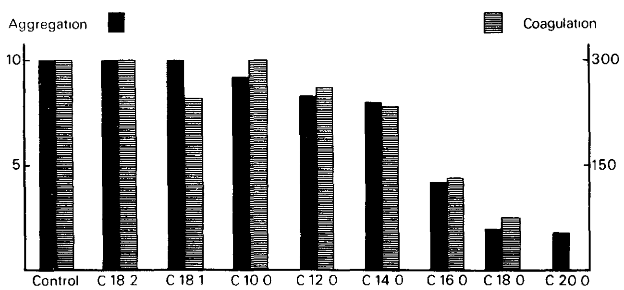


Fig. 6: Cholesterol and collagen content of the aorta in rabbit fed butter for different periods.

Concerning thrombosis, may I remind you that formation of a thrombus is the perturbation of a normal physiologic event called hemostasis, which normally stops the bleeding resulting from a wound or any damage to the integrity of the tissues.

As a thrombus, the formation of an hemostatic plus starts with aggregation of blood platelets, the smallest blood cell, and their adhesion to the vessel wall. In periphery of the platelet aggregates one can observe the formation of thin fibrin threads which will attach the platelet aggregate to the vessel wall and consolidate the fragile thrombotic scaffolding. A few hours later, platelet aggregates can still be observed, but fibrin becomes a major component of the thrombus. This schema appears to be similar in venous or arterial thrombosis.

One can ask whether the saturated fat intake predisposes to thrombosis and in particular, whether it affects the two main factors of a thrombus which are blood platelets and fibrin formation (clotting). In vitro studies in test tubes have shown that only the long chain saturated fatty acids (figure 7) but neither the short chain saturated fatty acids nor the mono or polyunsaturated fatty acids could cause platelet aggregation or initiate fibrin formation.



Adapted from Nossel H L .The contact phase of blood coagulation ,Blackwell Scient Publ 1964 Mahadevan .V et al Proc Soc Exper Biol Med 121 82 ,1966 , Conncr W E et al , Thromb Diath Haemorrh 13 89 ,1965

Fig. 7: Effects of fatty acids on coagulation and platelet aggregation in vitro (Renaud S. Dietary fats and thrombosis. Biblthca Nutr. Dieta 25: 92, 1977
Solely the saturated fatty acids with 16 or more carbon atoms have a highly significant effect on both coagulation and aggregation.

In addition, studies in rat fed different fats have shown that solely the saturated fatty acids with more than 14 carbon atoms could predispose to thrombosis, an effect apparently antagonized by linoleic acid, the main polyunsaturate fatty acid. It is the result which has been obtained by Hornstra (Hornstra, G. Dietary fats and arterial thrombosis, Haemostasis 2: 21, 1973) in Netherlands, which is essentially the result we obtained previously in a totally different system to this of Hornstra. In our system, stearic acid (18 carbon atoms) was the most thrombogenic acid, as it was in vitro on blood platelets and coagulation, since it was able to explain most of the thrombogenic properties of a number of dietary fats (figure 8).

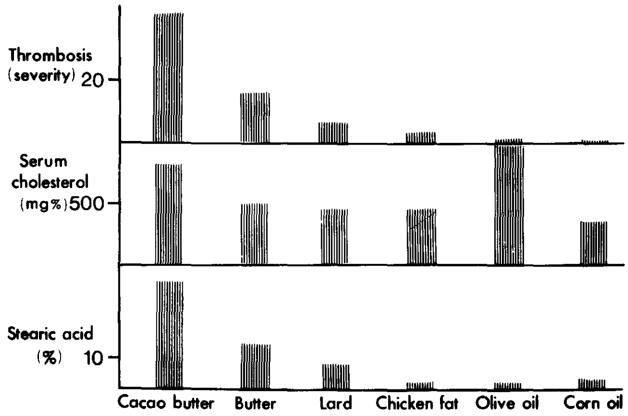


Fig. 8: Influence of common dietary fats on the production of thrombosis in the rat. The severity of thrombosis appears to be markedly related to the content of the fats in stearic acid (from Renaud, S. and Gautheron, P. Dietary fats and experimental (cardiac and venous) Thrombosis Haemostasis 2: 53, 1973).

Can we decrease or inhibit the predisposing effect of saturated fats by replacing them partly by polyunsaturated fats. For this purpose, in our experimental model in rats we have included in their diet either butter (38%) alone or butter (10%) to which was added 24% of one of the following commercial fats: a saturated margarine, palm oil, erucic rich rapeseed oil, hydrogenated coconut oil, corn oil, Primor low erucic acid rapeseed oil and a sunflower margarine (rich in linoleic acid) in such a way that fats supplied the same amount of calories in each group. The animals have been fed for a longer period than usual since the purpose of the study was not to determine what was the most thrombogenic fat but rather the fat that presented the most effective anti-thrombogenic properties. As shown in figure 9, only corn oil, Primor rapeseed oil and sunflower margarine presented a marked inhibiting effect on thrombosis as compared to butter. The anti-thrombogenic effect of these three fats was approximately identical, as was also similar their hypolipemic effect.

Nevertheless, as in most studies on thrombosis, there was no direct relationship between the thrombotic tendency and serum cholesterol since the saturated margarine was not much hypercholesterolemic, although it was responsible for the highest severity of thrombosis. Despite this, the lowest serum cholesterol was associated with the lowest thrombosis severity. Of interest is that the combination butter + rapeseed oil containing 28% erucic acid presented a thrombogenic effect intermediate between butter, and butter plus hydrogenated coconut oil, while no thrombosis could be observed with the feeding of butter plus Primor rapeseed oil (containing 0.2% erucic acid). In this system, as indicated in the figure, the hypercholesterolemic fatty acids appear to be myristic, palmitic, oleic, and erucic acid while linoleic and linolenic acids appear to antagonize this effect. This study indicates that from the point of view of cholesterolemia and thrombosis, erucic acid should be eliminated from rapeseed oil. Nevertheless, the intoward effects of erucic acid do not appear to be more damaging than those of a large number of fats for which there are not yet any plans to even descrease the production.

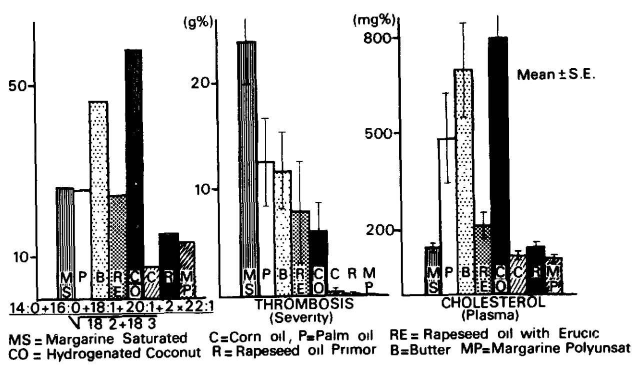


Fig. 9: Thrombogenic effects in rats, of commercial fats included in a hyperlipemic diet. 8 groups of animals were studied, the only difference being the type of fat included in the purified hyperlipemic diet given. The fats were either butter alone (38%) or butter (10%) plus one of the commercial fats (24%) listed in the figure. Solely corn oil, rapeseed oil Primor and the polyunsaturated (sunflower) margarine were able to completely counteract the thrombotic effect of butter. The effects of the fats on cholesterol in plasma appear to be related to the fatty acid composition of the dietary fats, more specifically to the following ratio 14:0 + 16:0 + 18:1 + 20:1 + 2 x 22:1/ 18:2 + 18:3.

We will rather now examine the application to man, of the experimental results observed in animals. This application has been started in 1976 with the study of farmers in two regions of France. In the two regions selected, the farmers were highly comparable from all points of views (owners of a farm, similar degree of physical activity, of responsibilities, of cigarettes smoked ...) except in the dietary habits, in particular the intake of saturated fats. The facts that the farmers take all their meals at home, that there is very little variation in their dietary habits from one day to another, that they have consistent cooking habits (utilization either of butter, saturated margarine or oil, cream or oil in the salad..), facilitate the dietary survey and reinforce its validity. In order to be able to study and sompare in farmers from various regions, the parameters involved in thrombosis, mostly blood platelets and coagulation, we had to organize a mobile-laboratory for the following reasons:

- the parameters mentioned above have to be studied in fasted subjects, in their habitat, in the minutes following blood removal;
- the sophisticated instruments utilized, have to be settled once and for all, and not shipped in the usual way in order to avoid any modification in the permanent settling. The mobile laboratory is a commercial trailer transformed into a laboratory in which each instrument (in duplicate in case of a breakdown) is tied to the bench through rubber blocks and connected to an electronically reulgated electric line. The trailer contains all the instruments needed for such studies (refrigerator, freezer, centrifuge, particle counters, aggrego- and coagulometers, electrocardiograph ...) in addition to the bed to determine the ECG at rest and make the blood removal. Two technicians can then perform the pultiple tests and prepare the samples (plasma, platelets, food aliquots) which are frozen for determinations performed in the main laboratory.

Comparative results between the two regions in the study performed in 1976 reproduce essentially the results observed in animals fed butter as compared to a vegetable oil. As illustrated in figure 10, only the animals fed butter, presented thromboses. A marked increase in the susceptibility of their platelets to aggregation (to thrombin) as well as an acceleration of blood clotting (fibrin formation) was also observed in these rats.

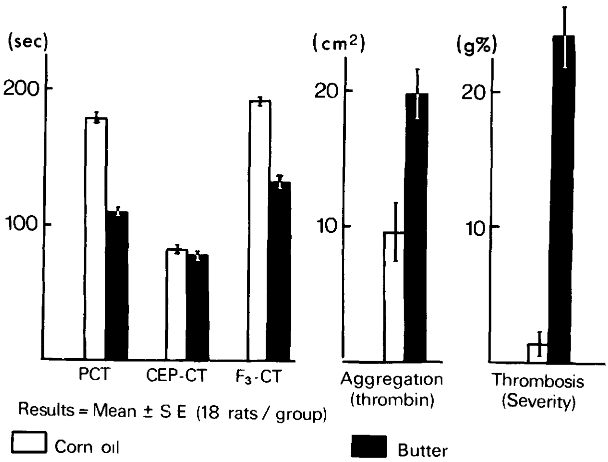


Fig. 10: Hematologic results in rats fed butter as compared to corn oil. Only the butter fed animals present thrombosis. This is related to a marked increase in thrombin induced platelet aggregation, and a highly significant shortening (acceleration) of the clotting (fibrin formation) time (PCT), not due to the plasmatic clotting factors (CEP-CT) but rather to the clotting activity of platelets (F_3-CT).

In farmers of Moselle as compared to those from Var, it is almost exactly what is observed in animals (figure 11) and the difference is somewhat similar to this obtained in animals fed butter as compared to corn oil. However, the cholesterolemia was the same in the two regions while the Official Statistics report a coronary heart disease incidence almost double in Moselle than in Var.

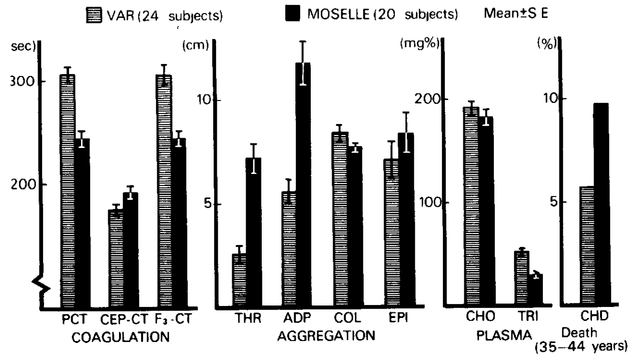


Fig 11: Blood parameters in healthy make farmers (40-45 years) from two French regions. Note the similarity of the results with those obtained in animals.

PCT: Recalcification clotting time of platelet rich plasma (PRP) (evaluates the clotting activity of whole blood)

CEP-CT: Cephalin clotting time of platelet poor plasma (evaluates the clotting activity of the plasmatic clotting factors)

F_3-CT: Clotting time of washed platelets, resuspended (400,000:mm^3) in a standard platelet poor plasma (evaluates the clotting activity of platelets).

The susceptibility of platelets to aggregation is evaluated by turbidimetry in PRP adjusted to 300,000 platelets/mm^3. The results are expressed as the maximal deflection curve in response to thrombin (THR), adenosine disphosphate (APD), collagen (COLL) and epinephrine (EPI). Cholesterol (CHO) and triglycerides (TRI) were evaluated in plasma.

As an indication, the incidence of mortality by coronary heart disease (CHD) is given (mean of 1970 yo 1972) for the two regions.

Concerning the differences in the dietary habits (figure 12), they are essentially at the level of the saturated fat intake, comparable in Moselle to that of North America, while in Var, it is similar to Italy or other Mediterranean countries.

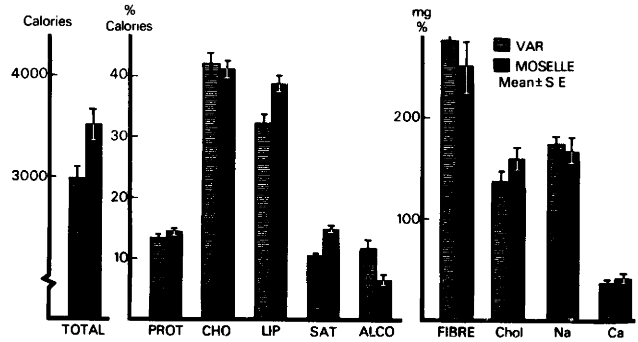


Fig. 12: Food intake composition of the farmers from the two regions, as resulting from the chemical analysis of a diet composite.
Proteins: PROT; Carbohydrates: CHO; Total Lipids: LIP; Saturated fats: SAT; Alcohol: ALCO; Cellulose: FIBRE; Cholesterol: Chol, Sodium: Na; Calcium: Ca. The most significant difference is in the saturated fat intake.

In the saturated fats, stearic acid appears to be able to explain, as in animals, most of the effects on blood platelets (figure 13) since it is correlated, on an individual basis, to the platelet functions as closely as the total saturated fatty acids (myristic + palmetic + stearic only, representing 90% of all the food saturated fatty acids). Of interest is that stearic acid is in quantity (approximately 25%), the second most important saturated fatty acid after palmitic acid (60%).

(% Calories)	F_3-CT	THROMBIN (0 03)	ADP (0 4)	CHOLESTEROL
ALCOHOL	0 06	-0.15	-0 27	0.30(p<0.05)
TOTAL LIPIDS	0 35 (p<0 05)	0 42(p<0.01)	0 31(p<0.05)	-0 15
MONOUNSATURATED	0 22	0 31(p<0.05)	0.22	-0 10
SATURATED	0 43(p<0.01) (0.44)*	0.59(p<0.001) (0 46)*	0.60(p<0.001) (0.57)*	-0.11
STEARIC	0 43(p<0 01)	0.60 (p<0 001)	0.50(p<0 001)	
POLYUNSATURATED	-0 07	-0 19	-0 30(p<0.05)	
PROTEINS	-0.06	-0 08	0.03	
CARBOHYDRATE	-0.35(p<0.05)	-0.13	-0.02	
FIBER (g/day)	-0.22	-0.04	-0.05	0.13

* Evaluated by the past recall and the dietary journal.

Fig. 13: Correlations (r) between certain blood parameters and food components. The saturated fat intake, and particularly this of stearic acid, is highly significantly correlated, on an individual basis, with the clotting activity of platelets (F_3-CT) and the aggregation to thrombin and ADP.

Because of the high intake of fat in Moselle, this region appeared to be ideal to set up a pilot study on the modification of the dietary habits for preventing cardiac diseases and, as a starting point, to improve the platelet functions. As shown in figure 14, 57 families (57 men and 22 women) from the Albestroff region, of which a part was covered in the 1976 study, have accepted to modify for one year, their dietary habits in such a way that they would become similar to those of Var. Another group of 50 families (50 men, 22 Women) in an adjacent region (Bitche), still in Moselle, do not modify their dietary habits and serve as controls.

1 — Subjects : Farmers

	Diet Modification		No Modification
	25 men 5 women	32 men 17 women	50 men 22 women
Type of fat	Margarine: Sunflower	Margarine: Sunflower+ Rapeseed Primor	Traditional (mostly butter, cream and saturated margarine)
	Oil: Sunflower+ Rapeseed Primor	Oil: Rapeseed Primor	
Other changes	Decreased intake of saturated fats from meat, delicatessen, and milk (2%)		—

2 — Two complete examination (EGG, blood tests, dietary survey) at one year interval (the first one, before diet modification if any)

Fig. 14: Moselle pilot study on the dietary habit modifications in relation to various blood parameters.

The dietary habit modifications can be simplified in the following way. Butter and cream were eliminated both in cooking and on bread, and were replaced by polyunsaturated margarine and vegetable oil; 2% skimmed milk was utilized and it was recommended to discard most of the fat from beef, lamb and pork. Concerning the fats supplied (figure 14), 32 families utilized sunflower + primor-rapeseed margarine (21% oleic acid; 49% linoleic acid, 1.2% linolenic acid and 0.4% erucic acid) and primor-rapeseed oil (57% oleic acid, 22% linoleic acid, 8% linolenic acid and 2% erucic acid). Twenty five families utilized sunflower margarine (18% oleic acid and 54% linoleic acid) and sunflower primor-rapeseed oil (41% oleic acid, 41% linoleic acid, 4% linolenic acid and 1.3% erucic acid). The composition in fatty acids of the fats is reported in figure 15. The purpose of this study divided in two groups was to try to determine to what extent it is necessary to increase the level of dietary linoleic acid for a same level of saturated fats, to obtain the most beneficial effect.

In addition, we wanted to determine whether mixing sunflower to primor-rapeseed oil could have advantage in practice.

		Margarine		Butter	Oil	
		Sunflower	Sunflower + Primor		Sunflower + Primor	Primor
Palmitic	(16:0)	11.0	10.1	37.0	5.8	5.4
Stearic	(18:0)	10.6	9.9	11.8	3.2	1.6
Oleic	(18:1)	18.4	21.0	23.5	40.8	56.9
Linoleic	(18:2)	53.5	49.0	1.8	42.0	22.2
Linolenic	(18:3)	0.1	1.2	0.8	3.7	8.1
Erucic	(22:1)	0.0	0.4	0.0	1.3	2.0

Fig 15: Main fatty acid composition of dietary fats in the Moselle study.

Concerning the psychologic and organoleptic effects of the dietary habit modifications with the fats utilized, the participants of this study have been asked, six months after the beginning of the diet modification, to answer a written questionnaire of which the essential is summarized in figure 16. From this, it seems, that the farmers did not have much difficulty in changing their diet pattern, that the margarine on bread or for cooking was evaluated as being good or excellent; that primor rapeseed oil for salads was highly appreciated while for frying, the mixture sunflower + primor was preferred.

- Difficult to change the dietary habits Yes : 9% No : 91%
- Difficult to change the cooking habits Yes : 2% No : 98%
- The taste :
- on bread of margarine Sunflower is excellent 20% good 80%
  Sunflower-Primor is " 3% good 94%
- of cooked margarine Sunflower is excellent 16% good 80%
  Sunflower-Primor is " 10% good 87%
- in salads of oil Sunflower-Primor is excellent 88% neutral 4%
  Primor is " 94% neutral 0
- of food cooked in oil Sunflower-Primor is excellent 76% neutral 12%
  Primor is " 45% neutral 39%

Fig 16: Psychologic and organoleptic aspects of margarine and oil utilisation.

Concerning the totality of the results (hematologic tests, lipemia ...) as compared to the controls, they will be available solely when the study will be completed, at the end of 1978. The only hematologic results available so far after one year, are on the first 18 subjects to have accepted modifying their diet. The main results as compared to those obtained one year before, are illustrated in figure 17. They indicate that replacing butter and cream by primor rapeseed oil and sunflower oil and margarine, decreases markedly the clotting activity and the aggregation (to thrombin) of platelets, the blood factor eventually the most closely associated with coronary heart disease.

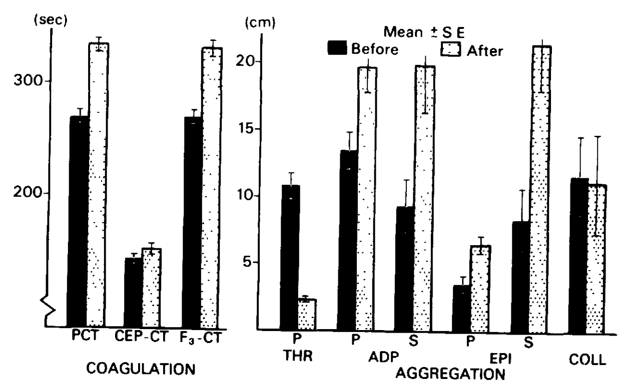


Fig. 17: Hematologic results in 18 male farmers from Moselte, before and one year after diet modification. Coagulation and aggregation to thrombin have been considerably modified and are similar now to the results observed in the Var. By contrast, a decrease in the susceptibility of platelets to ADP or epinephrine, was not observed during this period of time. These parameters seem to take more than one year to be modified.

CONCLUSIONS

- Concerning thrombosis, one of the two main components of coronary heart disease, the only important cardio-vascular problem in industrialized countries, we have shown that rapeseed oil rich in erucic acid presents untoward effects comparable to those of the most commonly used saturated fats.
- Primor rapeseed oil, low in erucic acid, prevents in animals, the butter-induced increase in cholesterol and predisposition to thrombosis to the same extent as corn oil or sunflower margarine.
- In man, primor rapeseed oil seems to have satisfactory organoleptic properties and appears to be able to normalize certain blood parameters related to coronary heart disease such as the clotting activity of platelets and their susceptibility to thrombin. However, definite results and the comparison between the beneficial effects of sunflower and those of primor oil will have to wait until the end of the present studies.

Symposium on new varieties of colza

EFFECT OF CERTAIN LOW-ERUCIC ACID COLZA OILS ON THE THROMBOSIS AND ATHEROSCLEROSIS FACTORS

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Brussels, 12 April 1978

SUMMARY

Studies carried out over a number of years in our laboratory in order to assess certain effects of fats in foodstuffs showed that the low-erucic acid oils tested here appear to have no unfavourable effect upon the thrombosis and atherosclerosis factors.

As far as thrombosis factors are concerned, the ingestion of Primor and Canbra oils appears even to have a favourable effect: these oils prolong the plasma coagulation time in man and animals without changing the platelet count or increasing platelet aggregation. Their modest linoleic acid content and their high oleic acid content make them similar from this point of view to olive oil, the "neutral" character of which in respect of platelet functions has already been underlined.

As to the atherosclerosis factors studied here, i.e. essentially lipidaemia, we have not been able to show significant differences between low-erucic acid colza oils and oils reputed to cause little hypercholesterolaemia, such as groundnut and sunflower seed oil. However, the essential alimentary factor is of a quantitative nature and it must be borne in mind that a diet too rich in fats may have adverse consequences whatever the type of fat ingested.

Finally, we found the effect of Canbra and Primor oils relatively favourable in respect of arterial lipids, particularly during one-year tests, which represents a weighty argument for emphasizing the harmlessness of these oils in relation to atherosclerosis.

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Over the last few years, we have undertaken at the request of the Inter-professional Technical Centre for Oil-bearing Plants grown in France, (CETIOM), a series of research projects connected with laboratory animals and in clinics in order to evaluate the consequences of ingesting different alimentary fats on the atherosclerosis and thrombosis factors.

The studies bore in particular on certain low-erucic acid colza oils. Apart from certain fats such as butter, olive oil, groundnut oil and sunflower seed oil, which served as reference oils, three types of colza oil were studied : high-erucic acid colza oil (37%) containing also high gadoleic acid (8.4%), Canbra (Canadian Brassica) oil for which the erucic and gado-leic acid levels are very low, and Primor oil, a French variety, which has also very low levels of erucic and gadoleic acids.

The latter two oils were characterized by their high percentage of oleic acid (respectively 63 and 56%), a linoleic acid level of about 20% and a fairly high level of linolenic acid (respectively 7 and 10%).

In the various projects which we present here, we have applied ourselves to studying essentially the effect of ingestion of these different fats on the factors which play a role in the development of atherosclerosis and thrombosis. The reason for this was the development of lesions of atherosclerosis and thrombosis, which gives rise to serious ischaemic diseases : myocardial infarction, angina pectoris, arteritis of the limbs, cerebral vascular damage. These diseases between them account for nearly half the deaths in developed countries.

Low arterial irrigation of an area, i.e. ischaemia, is the result of two associated processes : atherosclerosis and thrombosis.

TABLE I

FATTY ACID COMPOSITION OF 3 COLZA OIL VARIETIES STUDIED

Fatty acids / Oils	C_{16}	$C_{16'}$	C_{18}	$C_{18'}$	$C_{18''}$	$C_{18'''}$
COLZA	4,2	0,7	1,1	24,4	17,3	6,2
CANBRA	4,4	0,2	1,3	63,2	21,4	7,0
PRIMOR	4,6	0,1	1,6	55,6	19,0	10,0
	C_{20}	$C_{20'}$	C_{22}	$C_{22'}$	C_{24}	
COLZA	0,3	8,4	-	37,4	-	
CANBRA	0,3	1,4	-	0,3	-	
PRIMOR	0,8	2,1	0,4	0,5	0,5	

Atherosclerosis develops very slowly along the walls of the large and medium-sized arteries, narrows the artery, reduces the suppleness and elasticity of the wall and favours the development of aneurysms. This takes place over years or even decades.

Thrombosis is an abrupt lesion due to the formation of a clot inside the artery which obstructs more or less completely the arterial aperture.

These two processes are indissociable from one another, thrombosis appearing generally at the level of atherosclerosis lesions and seriously aggravating the circulatory situation in their neighbourhood.

However, the two processes, atherosclerosis and thrombosis, do not have identical causes. Factors in atherosclerosis are hypercholesterolaemia, high arterial blood pressure, diabetes, sedentary habits and heredity. Factors in thrombosis are fall in arterial flow, of whatever cause, nicotinism, oral contraceptives, all the causes of arterial endothelial lesions and the causes of the increased adhesion and aggregability of blood platelets.

Alimentary fats contribute in a complex way to these two processes which give rise to ischaemia of the "downstream" area.

In the case of atherosclerosis, it is essentially by means of the blood lipids and particularly blood cholesterol. It is well known that excessive consumption of fats increases the cholesterol content of the blood and this is true whatever the type of fat ingested, whether saturated or polyunsaturated. An excess consumption of glucides also increases the lipid content of the blood. There exists finally in certain subjects a defect, which is often congenital, of the lipid metabolism, which leads to hypercholesterolaemia and it is in this case that it is useful not only to reduce the total intake of fat calories, but also to pay attention to their quality, as certain polyunsaturated fats appear in such cases to have a favourable action.

As far as the thrombosis process is concerned, the role of the alimentary fats appears better established : it is clear that saturated fats increase the aggregation of platelets while certain polyunsaturated fats decrease the platelets aggregation. But here again the quantity of fat ingested is important, with the risk varying according to the individual.

It can be seen that such a study is complex and that it is difficult to identify the role of each factor, particularly food factors, in the development of ischaemic diseases. The problem is further complicated by the absence of a good animal model and by the difficulty of assessing arterial lesions in a living subject.

EXPERIMENTAL STUDIES

In the experimental work which we present here on colza oils, we have assessed the effect, under different conditions, of diets differing as to the nature and the percentage of fats they contain and the length of administration. The rat was chosen, even though it only develops atherosclerosis with great difficulty, since it represents a satisfactory and convenient model for the study of blood factors, notably hypercholesterolaemia and platelet aggregation.

In short-term experiments, after one month of a fat-rich diet (35% fats in the diet), we were able to show that the cholesterolaemia does not differ significantly whether the animals fed on colza, Canbra, groundnut, sunflower seed and Primor oil or on butter.

On the other hand, the animals fed on butter have a significantly higher triglyceridaemia than the animals fed with the other fats.

The level of cholesterol in the aorta does not differ significantly according to the different diets. The level of hepatic cholesterol is higher among the animals fed on butter.

The level of cardiac cholesterol is significantly higher in the animals fed on high-erucic acid colza, while those fed on Canbra and Primor have a cardiac cholesterol level which does not differ significantly from that of the animals fed on butter, groundnut or sunflower seed oil.

A study of the serum and arterial fatty acids was made in one experiment by comparing the groundnut, sunflower seed and Primor diets. Multiparametric analysis of the results (analysis of correspondences according to Benzecri) showed that the free fatty acids and the triglycerides of the animals fed on sunflower seed oil were very rich in linoleic acid and that this was true both for the serum and the aorta. On the other hand, a very high level of cholesterol laurate is noted in the aortas of animals fed on groundnut or sunflower seed oil, while this level is low in animals fed on Primor. This may be regarded as a point in favour of Primor oil.

Two long-term studies, lasting for a year, were carried out on the rat. In one, we compared the following fats : butter and groundnut, sunflower seed, colza, soya, hydrogenated soya, palm and Canbra oils. In the second, three oils with low levels of saturated fatty acids were compared : groundnut, sunflower seed and Primor.

In the two experiments, the quantity of lipids in the diet was low (6% of the total weight of the feedingstuff, i.e. 12.5% of the calorific value).

In the first experiment, we found significant differences between the lots of animals in respect of cholesterolaemia (higher with palm and hydrogenated soya oils, lower with sunflower seed and groundnut oils and intermediate with colza, Canbra and soya); as regards the triglyceridaemia, the values were higher for butter and palm oil, lower for groundnut and sunflower seed and intermediate for colza, Canbra and soya. In respect of aortic cholesterol, the highest values were for those fed on soya and hydrogenated soya and the lowest were for Canbra and butter.

The second long experiment comparing groundnut, sunflower seed and Primor oils showed no difference in respect of cholesterolaemia, triglyceridaemia or the level of arterial cholesterol. Multiparametric analysis of the fatty acid composition of the serum and arterial lipids showed no difference between the three lots except for cholesterol esters in the aorta, which contained more cholesterol laurate in the sunflower seed lot. This result thus confirms the short-term experiment.

In all these experiments, whether of short duration with a diet containing 35% fat, or of long duration with 6% fat, coagulation and platelet aggregation tests were performed. The following conclusions may be derived from these :

TABLE II

COAGULATION TIME IN RATS ON THE LONG-TERM DIET

	Coagulation time (CT) in seconds	Number of animals	Difference in CT Butter/Oil (*)
Butter	330 ± 13	7	NS
Hydrogenated soya	336 ± 17	9	NS
Colza	353 ± 23	9	NS
Soya	362 ± 30	9	NS
Groundnut	370 ± 28	7	NS
Palm	385 ± 19	8	$P < 0{,}05$
Sunflower seed	393 ± 19	8	$P < 0{,}05$
Canbra	455 ± 17	8	$P < 0{,}01$
(*) t-test			

The plasma coagulation time is the shortest with animals fed on butter and hydrogenated soya, and longest with animals fed on Primor, Canbra and sunflower seed. There is no difference in the platelet count between the various lots. The platelet aggregation is correlated with the level of linoleic acid in the diet, which confirms the data of various other authors.

In a five-month experiment on rabbits comparing three fats, butter, olive oil and Primor oil, the two oils stood out noticeably against butter since the platelet aggregation in the animals receiving the oils was reduced in comparison to those fed on butter.

TABLE III

RABBIT EXPERIMENT B : PLATELET AGGREGATION

	BUTTER (4 rabbits)	OLIVE (5 rabbits)	PRIMOR (3 rabbits)	Difference B/O	Difference B/P	Difference O/P
Rate in %	41,4 ± 2,6	32,3 ± 1,0	35,7 ± 3,2	<0,01	N.S.	N.S.
Intensity 1mn in %	50,0 ± 3,2	41,0 ± 1,8	40,1 ± 3,5	<0,02	<0,05	N.S.
Intensity 2mn in %	65,8 ± 5,5	52,1 ± 2,1	51,9 ± 2,5	<0,02	<0,05	N.S.
Intensity 3mn in %	73,3 ± 8,0	54,3 ± 1,6	56,8 ± 1,9	<0,01	<0,01	N.S.
Rate in %	37,9 ± 3,2	27,1 ± 2,1	29,1 ± 4,2	<0,05	N.S.	N.S.
Intensity 1mn in %	48,2 ± 2,3	35,9 ± 3,5	38,8 ± 3,9	<0,02	N.S.	N.S.
Intensity 2mn in %	59,4 ± 3,6	44,3 ± 3,0	47,9 ± 2,0	<0,02	<0,05	N.S.
Intensity 3mn in %	69,9 ± 1,1	45,7 ± 2,9	51,2 ± 2,1	<0,01	<0,01	N.S.

Same experiment as Table I. The result of the platelet aggregation tests are as a percentage of the theoretical maximum result.
The differences were determined by the t-test.

CLINICAL TESTS

Canbra oil and butter were compared using normal human subjects in a short clinical test lasting five days in order to assess the effects upon lipid metabolism, the coagulation tests and the platelet functions.

The diet administered for five days contained 90 g of the test fat divided between three meals, i.e. 810 calories, plus 480 calories in the form of protein and 900 calories in the form of carbohydrates. On the morning of the fifth day, the subjects were put through an induced hypervitaminaemia A test. The total cholesterol and triglycerides were measured. The platelets were counted, the plasma coagulation and the cephalin-kaolin times were measured and an in vitro study was made of the aggregation with ADP.

The result of the induced hypervitaminaemia A test did not appear significantly different for the two diets, although the serum vitamin A values had been definitely lower at the third and the sixth hour after the Canbra diet.

The mean level of serum cholesterol was lower for the Canbra diet than for the butter diet ($p<0,05$), while there was no difference as to the triglycerides.

The plasma coagulation time was slightly prolonged in subjects ingesting Canbra oil but the difference did not attain a statistically acceptable level.

The cephalin-kaolin time was not different for the two groups.

There was no significant difference in the platelet count. The platelet aggregation was more pronounced with the butter than with the Canbra oil diet. The differences observed here are significant for the rate at the three concentrations of ADP used, for the intensity at 1 minute at concentrations of 1,2 and 0,6 μM in the final solution; finally for the intensity at 2 mn at the concentration of 1,2 μM in the final solution. With solutions low in ADP concentration, one finds with the majority of subjects a platelet disaggregation.

This was complete in 2 mn with the concentration of 0,3 μM in all the subjects of the Canbra lot, and in 11 out of 12 subjects of the butter lot.

TABLE IV

	BUTTER	CANBRA	Significance
Howell time (in secs)	127 ± 26 (11)	144 ± 13 (11)	NS
Cephalin-Kaolin time (in secs)	68 ± 29 (10)	80 ± 7 (6)	NS
Platelet count mn^3	428.000 ± 137.000 (11)	377.000 ± 75.000 (11)	NS
Platelet aggregation rate %	49,7 ± 10,8 (12)	36,10 ± 5,6 (10)	$p < 0{,}01$
Intensity (%) at 1 mn	53,7 ± 6,0 (12)	45,7 ± 7,8 (10)	$p < 0{,}02$
Intensity (%) at 2 mn	67,7 ± 8,3 (12)	54,5 ± 13,8 (10)	$p < 0{,}02$

CLINICAL STUDY

Coagulation time, platelet count and platelet aggregation as measured on the fifth day of the test. The figures shown are the average standard deviation and the number of subjects (in brackets).

The significance was determined by the t-test (comparison of averages)

CONCLUSIONS

Studies carried out over a number of years in our laboratory in order to assess certain effects of fats in foodstuffs showed that the low-erucic acid oils tested here appear to have no unfavourable effect upon the thrombosis and atherosclerosis factors.

As far as thrombosis factors are concerned, the ingestion of Primor and Canbra oils appears even to have a favourable effect: these oils prolong the plasma coagulation time in man and animals without changing the platelet count or increasing platelet aggregation. Their modest linoleic acid content and their high oleic acid content make them similar from this point of view to olive oil, the "neutral" character of which in respect of platelet functions has already been underlined.

As to the atherosclerosis factors studied here, i.e. essentially lipidaemia, we have not been able to show significant differences between low-erucic acid colza oils and oils reputed to cause little hypercholesterolaemia, such as groundnut and sunflower seed oil. However, the essential alimentary factor is of a quantitative nature and it must be borne in mind that a diet too rich in fats may have adverse consequences whatever the type of fat ingested.

Finally, we found the effect of Canbra and Primor oils relatively favourable in respect of arterial lipids, particularly during one-year tests, which represents a weighty argument for emphasizing the harmlessness of these oils in relation to atherosclerosis.

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Symposium on new varieties of colza

EFFECTS OF NEW CRUCIFER OILS ON THE MYOCARDIUM: HISTOMETRIC STUDIES ON VARIOUS ANIMAL SPECIES

R.O. Vles,
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Vlaardingen, Netherlands.

Brussels, 12 April 1978

SUMMARY

Detailed morphometric studies on the myocardium of Wistar rats, mice, pigs and rabbits show the harmlessness of rapeseed oils with low erucic acid contents.

The great sensitivity of the male rat of the Sprague-Dawley strain to cardiac necrosis is confirmed. The linolenic/linoleic acid ratio and the relative excess of monounsaturated fatty acids which characterise the oils of the new varieties of rapeseed explain the phenomena observed in this particular experimental model.

The existence of a specific, non-triglyceride antinutritional factor in cruciferous oils is improbable.

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INTRODUCTION

A considerable amount of work has been done on the nutritional and physio-pathological effects of high-erucic acid cruciferous oils. In these studies, performed on a great variety of animal species, various aspects have been considered, e.g. the growth curve, mortality and longevity, feed conversion, state and functions of the different organs, resistance to stress, muscular contractility and cellular and subcellular biochemistry. The results obtained have shown that the development of new varieties of rapeseed with little or no erucic acid is a considerable step forward in nutrition.

However, certain reservations have been expressed in view of the fact that the prolonged administration of low-erucic acid rapeseed oils still caused cardiac lesions, particularly in male rats, although these lesions were less frequent, less severe and appeared later than those observed after administration of high-erucic acid oils.

These "residual" abnormalities raised a problem of interpretation because the detailed and systematic examination of a large number of hearts of control animals revealed similar lesions. The lack of specificity in this type of defect, combined with their very variable frequency from one experiment, strain or species of animal to another, made a quantitative investigation essential.

It was therefore the existence of a background incidence of myocardial lesions which led us to adopt a standardized histometric methodology.

We propose to describe briefly the technique used. After this, the results obtained on the various animal species studies will be presented. Finally, we will explain how closely the information obtained enables the nutritional status of the new crucifer oils to be defined.

HISTOMETRIC TECHNIQUE

The technique consists, firstly, of taking specimens by a method which varies somewhat according to the species of animal studied. Histological sections of a thickness of 5 microns, cut across the main axis of the cardiac muscle are then stained and coded. Giving a code number enables the microscope examination to be made "blind", i.e. without prior knowledge of the respective treatments. The equipment used for the qualitative and quantitative examination is shown schematically in Fig. 1. It consists of a device for microphotographic projection provided with a scanning stage with programmed movement. The programming is carried out by a microprocessor. In addition, the microprocessor or auxiliary memory records all the observations and reproduces them with the aid of a teletype device. A control panel and a joystick complete the apparatus.

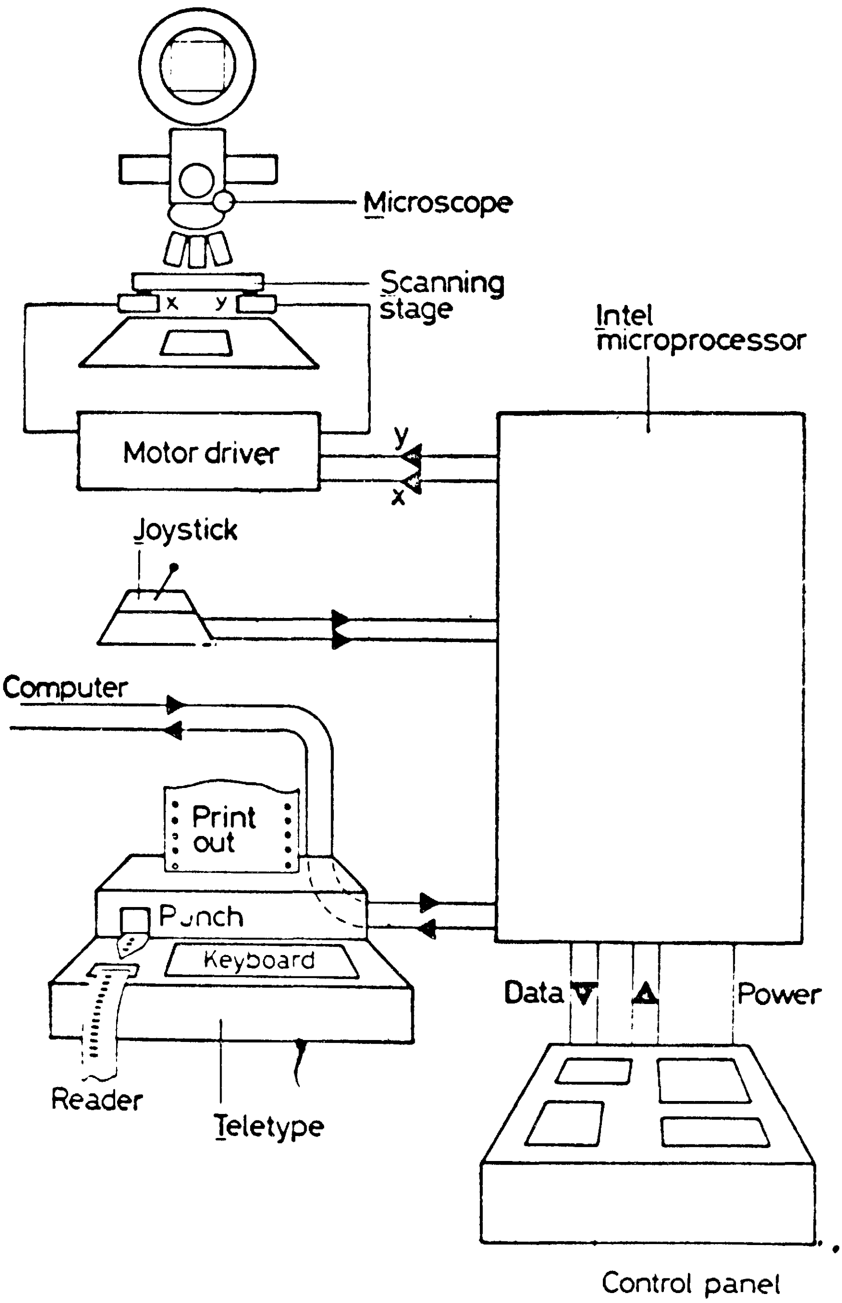

Figure 1 : Analysis technique

The histological slide, moved along two axes (x,y) perpendicular to each other is stopped when an abnormality appears on the screen. The operator types the lesion, according to whether there are interstitial or granulomatous cellular infiltrates, areas of lysis with loose sclerosis, scars or mutilating fibrosis.

All lesions, with the exception of diffuse interstitial infiltrates, are measured with the aid of a millimetre scale, the measurement taken being the Feret statistical diameter (horizontal projection of the lesion surface). The numbers and types of lesions, their Feret diameters and their locations (x and y coordinates) are, after recording and reproduction, available for statistical analysis.

The "number of animals affected" criterion was submitted to the x^2 test. The data referring to the number and the extent of the lesions showed a non-normal distribution and this is why the statistical analysis, in this case, made use of a non-parametric method, i.e. Kruskal-Wallis method of analysing the variance of observations ranked on increasing order of severity. Severity is defined both by the total number of lesions per animal, at the various levels examined, and by the individual sum of the Feret statistical diameters.

Using this methodology, we examined a considerable number of hearts from different species and strains of animals kept on various diets for varying times.

EXPERIMENTS WITH RATS

In the rats, the morphometric study essentially concerned a wide median part of the myocardium. The transversal microscopic sections were sampled at five equidistant levels, the distance between sampling levels being 0.8 mm (Fig. 2).

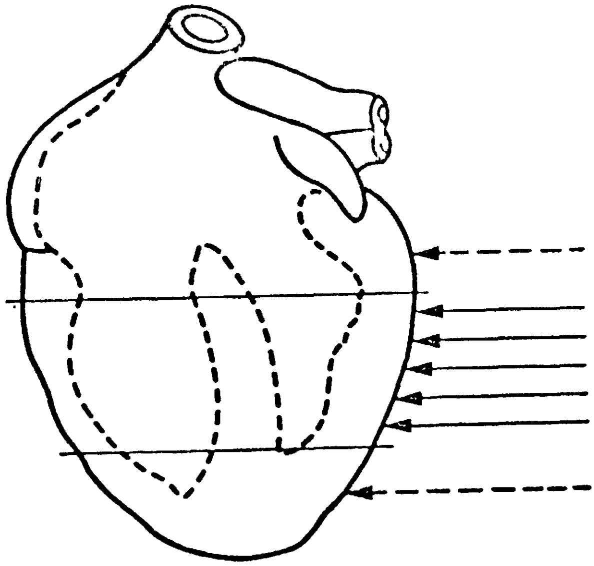

Fig. 2 : Sampling levels in rats

An initial experiment with Wistar rats (strain Cpb-WU) enabled us to confirm the long-term cardiotoxic role of erucic acid. In this test, three oils were compared: sunflower oil, a Canadian oil ("double zero colza", from seed with low erucic acid and glucosinolate contents) and conventional high-erucic acid rapeseed oil. As an account of the experiment and its results have been published elsewhere, only the conclusions of the statistical analysis of the histomorphometric data will be presented (Table I).

Table I: Statistical analysis of histometric data: Six-month experiment with Wistar rats (n = 12)

Criterion	Classification in decreasing order of severity		
No of animals affected	Ordinary colza	Colza 00	Sunflower
No of lesions per animal	Ordinary colza	Colza 00	Sunflower
Sum of Feret diameters	Ordinary colza	Colza 00	Sunflower

The continuous line under the different treatments indicates that there is no significant difference. Conversely, each gap indicates a significant statistical difference.

Table I confirms what a large number of authors claim - that erucic acid causes a significant increase in both the frequency and the gravity of myocardial lesions, which however are not infrequently found in control animals.

Using the same strain of rats and studying this time the effect of increasing quantities of Primor oil in diets with 40% of their energy value as oils and fats, we found no relation between the dose of Primor (crucifer oil without erucic acid) and the severity of myocardial lesions, taking into account the different types of lesions, their number and their size (Table II).

Table II: Statistical analysis of histometric data. Six-month experiment on Wistar rats subjected to increasing doses of Primor oil from 0 to 40% of the energy value n = 16)

Criterion	Classification, in decreasing order of lesions severity, of doses of Primor administered (% energy)				
No of animals affected	40	0	5	20	10
No of lesions per animal	0	40	5	20	10
Sum of Feret diameters	0	40	5	20	10

The continuous line under the different treatments indicates the absence of any statistically significant differences

Thus these studies, and others, do not show up any particular disadvantages in feeding animals on oils from new cruciferous varieties.

Some reservations have nevertheless been expressed based on anatomical studies on the male rat showing differences between low-erucic acid oils and other edible fats and oils. Histological examinations seemed to indicate considerable differences in sensitivity to cardiac necrosis between the strains of rats used. This is why we decided to study, by means of our quantitative technique, the hearts of two strains of rats under rigorously comparable experimental conditions.

To this end we administered simultaneously, to groups of Wistar and Sprague-Dawley rats, high quantities of Primor oil, linseed oil, olive oil and sunflower oil for periods of six or 12 months. It is known that the linseed, olive and sunflower oils are rich sources respectively of linolenic, oleic and linoleic acids, while Primor oil combines a very high level of oleic acid with a particular linolenic/linoleic acid ratio.

The results of the morphometric study can be summarized in three histograms. The first histogram (fig. 3) shows that as absolute values the differences between the Wistar (W) and the Sprague-Dawley (S-D) strains are, when all is said and done, greater than the differences between feeding treatments, with the single exception of sunflower oil.

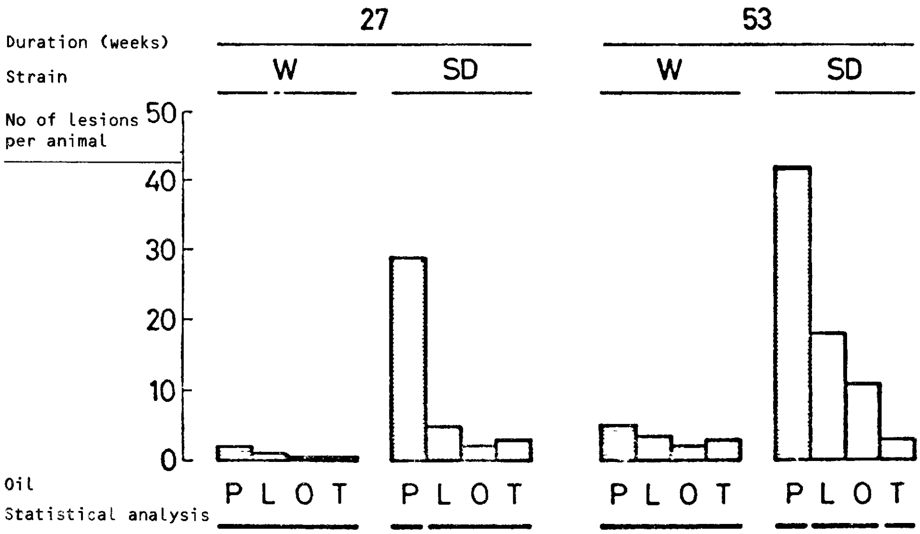

Fig. 3: Effects of Primor oil (P), linseed oil (L), olive oil (O) or sunflower seed oil (T) on the myocardium in the rat (n = 18)

The statistical analysis of results obtained on Wistar rats (Fig. 4) indicates that there is hardly any difference from one oil to another, thus confirming our earlier data.

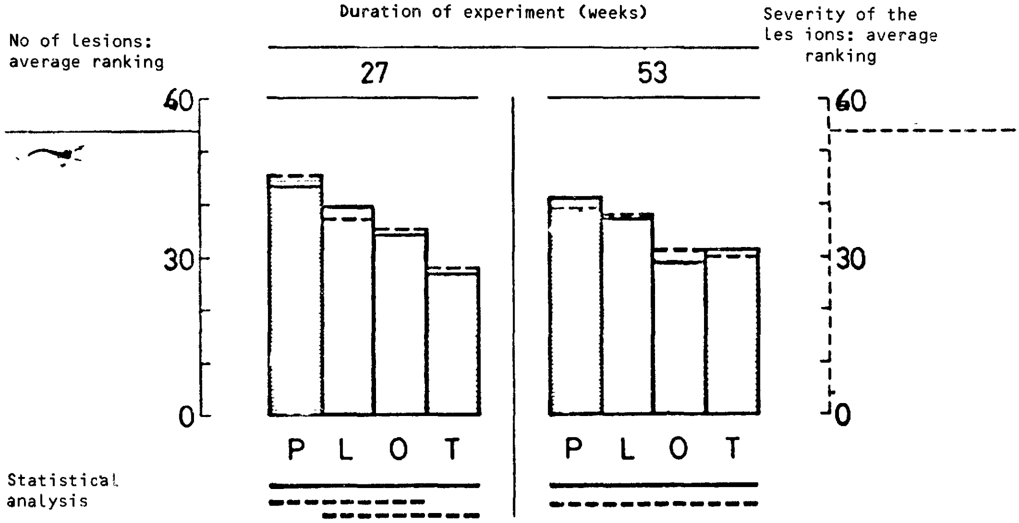

Fig. 4: Effects of Primor oil (P), linseed oil (L), olive oil (O) and sunflower oil (T) on the myocardium: Wistar rats (n = 18, 40% energy)

Figure 5 shows the results on Sprague-Dawley rats. The dotted lines in the histograms relate to the severity of the lesions as defined by the sum of the Feret diameters. The results on Sprague-Dawley rats show that both after six months and after one year the rats of this strain clearly develop more lesions on Primor oil than on a diet containing linseed, olive or sunflower oil.

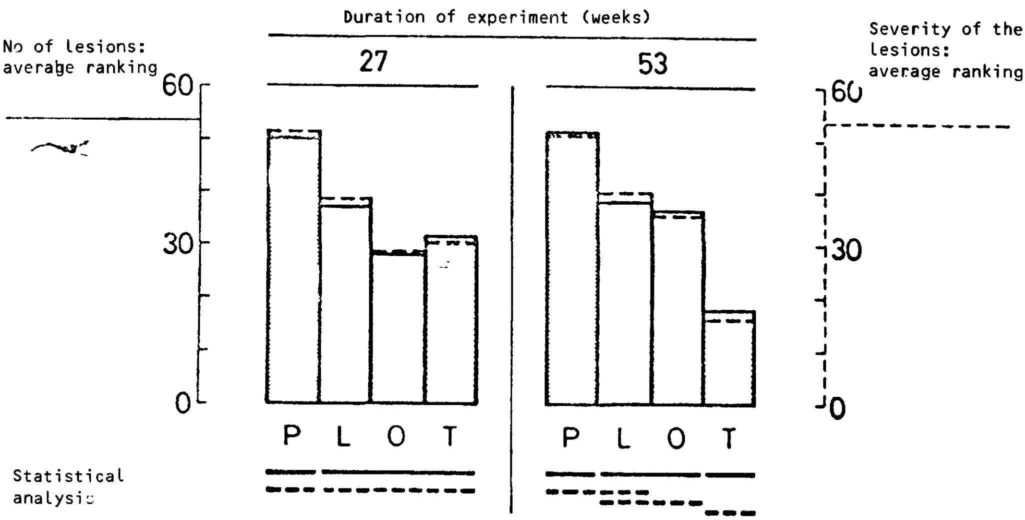

Fig. 5: Effects of Primor oil (P), linseed oil (L), olive oil (O) and sunflower oil (T) on the myocardium: Sprague-Dawley rats (n = 18; 40% energy)

The part of the histogram representing the relative order after one year indicates in addition a distinctly favourable effect with sunflower oil as against olive or linseed oil.

In the case of Primor oil, the interaction between oleic acid and linolenic acid appears to be responsible for its less favourable classification. We believe that limiting the combined amount of linolenic and oleic acid ought to be sufficient to reduce the differences observed.

We shall list the factors which argue for the need to rebalance a diet with a relatively uniform fatty acid content.

We shall refer to the result of the INRA experiments already presented by Mr. Flanzy. We shall then say a word or two about research carried out in collaboration with Professor Mangold of the Federal Centre for Lipid Research in Münster. We will mention research showing that the residual effects disappear with mixing or dilution, and finally we shall describe the results observed in other animal species.

Using the technique described above, we examined material from an INRA experiment in which rats received diets containing variable quantities of groundnut or Primor oil. Figures 6 and 7 summarize the results of the histometric study.

There are no marked differences between groundnut oil and Primor oil although there is a tendency towards more fibrosis lesions at 15% Primor, an effect which one might relate to the presence of linolenic acid in this diet.

The only differences which are definitely significant are those between the 10 and 15% levels of lipids, irrespective of which oil was given (Fig. 6).

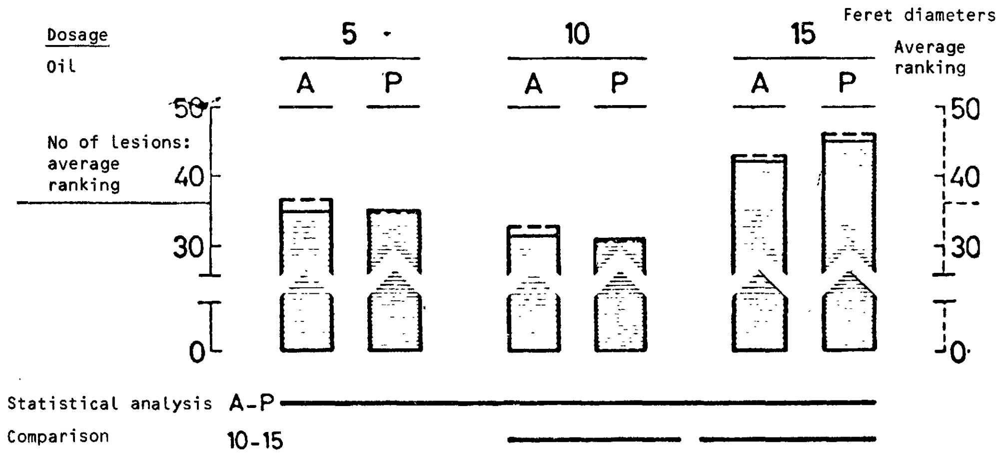

Fig. 6: Effects of groundnut oil (A) or Primor oil (P) on the myocardium: INRA rats (n = 12, 6 months)

The second figure, relating to INRA material, confirms that after one year there is no difference between 5% groundnut oil and 5% Primor oil (Fig. 7). Indeed, it gives information on what one is tempted to call the base-line pathology of the material studied.

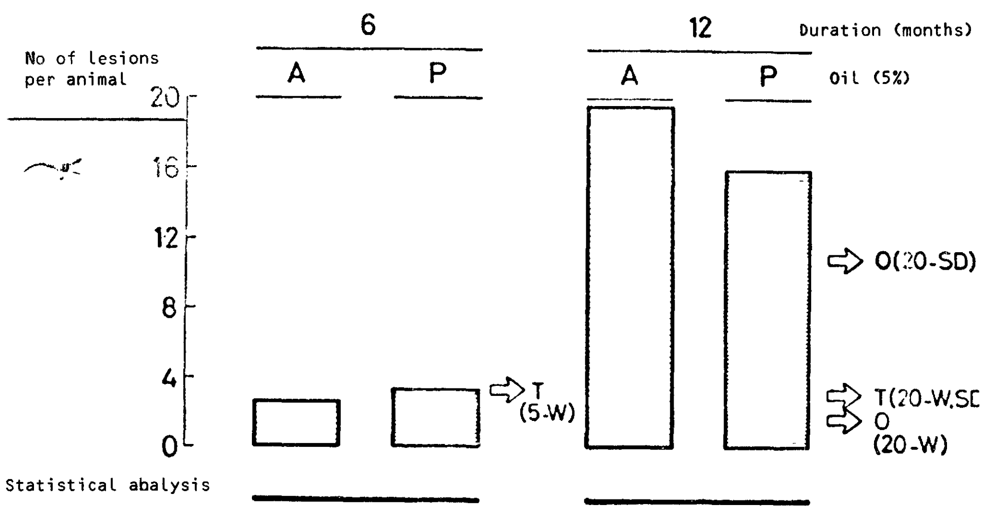

Fig. 7: Effects of groundnut oil (A) or Primor oil (P) on the myocardium: INRA rats (n = 12)

It is found that the number of lesions has increased five-fold after one year compared with six months, whatever oil is used.

With the aid of arrows (Fig. 7) we have added some of our earlier data to that derived from the INRA animal material. It can be seen that the base-line pathology of INRA rats lies at about the level of our Sprague-Dawley rats fed on 20% by weight of olive oil. On the other hand, our rats fed on sunflower oil, whether they be Wistar or Sprague-Dawley strain, are characterized by singularly slow cardiac ageing. In evaluating the possible cardiopathogenic effects of any particular oil, it is important to take into account the indisputable cardioprotective effect of sources rich in linoleic acid, particularly for strains sensitive to cardiac necrosis.

The possibility of there being a non-glyceride cardiotoxic factor in cruciferous oil is often mentioned. The studies which we carried out in close collaboration with the Federal Centre for Lipid Research in Münster, which will be presented at the Fifth International Congress on Rapeseed at Malmö next June, have not enabled us to show any cardiopathogenicity in either the unsaponifiable or the other fractions obtained from rapeseed. Thus, even when increased five-fold over its level in the original oil, the unsaponifiable fraction still had no particular effects on the hearts of rats. The same was true for a concentrated fraction of glucosinolates.

These results agree with Canadian and French experiments showing that there is little likelihood that the non-lipid or non-glyceride components of rapeseed oils may be regarded as suspicious.

The third argument for the concept of readjustment of the fatty acid balance, as we have already mentioned, is the disappearance of all pathogenic effects when the new low erucic acid oils are given in mixtures with other sources of feed lipids. This suppression of the antinutritional effect by plending is rarely observed when Sprague-Dawley rats are given high erucic acid cruciferous oils.

Finally, and this is the main point, if the probability of a risk for human beings was considerably increased by ten and more animal species being adversely affected by high erucic acid rapeseed oil, the same does not apply to rapeseed oil without erucic acid.

The histometric studies carried out on mice, rabbit and pig hearts make the extrapolation to man of the results observed on Sprague-Dawley rats very hazardous.

Before describing recent research on various other species in detail, we will list the different publications which confirm the superiority of low erucic acid rapeseed oil compared with conventional ones. Table III gives the bibliographic references for the periods 1973-75 and 1975-77.

TABLEAU III

REFERENCES TO EXPERIMENTS SHOWING NO ADVERSE EFFECTS OF LOW-ERUCIC-ACID RAPESEED OILS

1973-1975		
PIGS	Friend et al;	Can. J. Anim. Sci. 55. 49 (1975)
PIGS	Aherne et al;	Can. J. Anim. Sci. 55. 77 (1975)
CHICKENS	Lall and Slinger	Poultry Sci. 52 1729 (1973)
CHICKENS	Voogtman and Clandinin	Poultry Sci. 53 2108 (1974)
DUCKLINGS	Abdellatif and Vles	Poultry Sci. 52 1932 (1973)
1975-1977		
CYNOMOLGUS MONKEYS	Bio-Research study	R.U.A.P.CANADA (1975)
M. FASCICULARIS	Loew et al;	in preparation (1977)
PIGS	Friend et al.	Can J. Anim. Sci. 56. 361 (1976)
	Aherne et al.	Can J. Anim. Sci. 56. 275 (1976)
CHICKENS	Ratanasethkul et al.	Can. J. Comp. Med. 40. 360 (1976)
	Clement and Renner	J. Nutr. 107 251 (1977)
DUCKLINGS, TURKEYS	Ratanasethkul et al.	Can. J. com. Med. 40. 360 (1976)
SWISS MICE	Vles et al.	Fette Seifen Amstrichn. 78 126 (1976)

HISTOMETRIC STUDIES ON OTHER SPECIES

By carrying out on mice practically identical experiments to those done on rats, we obtained the results shown in Table IV.

Table IV: Myocardium lesions in mice subjected for 5 months to a diet containing 40% energy value in the form of Primor oil, olive oil, linseed oil or sunflower oil

Diet		Primor	olive	linseed	sunflower
Criteria					
No of animals	examined	28	26	24	26
	affected	0	5	2	1
No of lesions per animal		0	0.4	0.1	0.1
Mean sum of the Feret diameters (in mm 160 x)		0	11.3	2.8	3.2

The examination of 560 histological sections from 28 mice which had received 40% of their energy value in the form of Primor oil for 5 months shows a completely intact myocardium. The results demonstrate that Primor is not harmful to mice, whereas diets high in erucic acid have been shown to be harmful.

The third species subjected to prolonged diets rich in Primor oil was the rabbit. We subjected the animals for two years to semi-synthetic diets with 40% of their energy value in the form of Primor oil, palm oil or sunflower oil.

Rabbits are often used in cardiovascular pathology since, providing certain precautions are taken in experiments, they develop atherosclerotic lesions very similar to human vascular lesions. In addition, it is not uncommon to find small foci of myolysis and fibrosis in the myocardium. The histometric study was carried out at six levels of sampling, which are shown in Fig. 8.

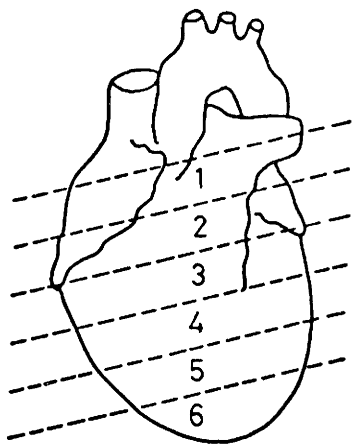

Fig. 8: Sampling levels in Rabbits.

Macroscopic examination of the aorta and morphometric study of the myocardium allow two conclusions to be drawn. As regards cardiac muscle, there is no difference between the diets (Fig. 9).

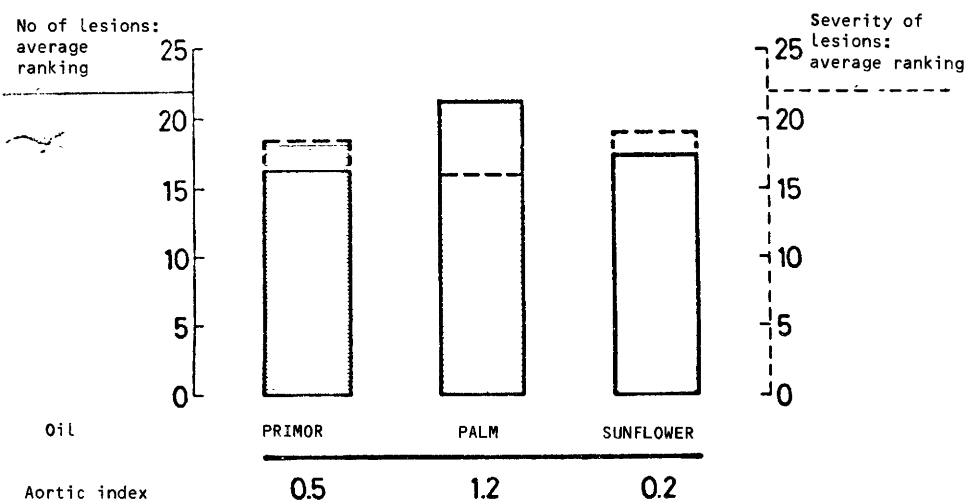

Fig. 9: Effects of diets on the myocardium: two-year experiment with rabbits (n = 9 - 13. 40% energy)
As regards atherosclerotic lesions of the aorta (aortic index)

As regards atherosclerotic lesions of the aorta (aortic index), the Primor diet has a certain protective effect compared with palm oil. This can be related to the quantity of linoleic acid present in the diet. The antiatherogenic effect of alimentary linoleic acid is thus confirmed.

Finally, still using the same methods, we studied the hearts of pigs. These animals had been subjected for four months to different diets. The animal husbandry part of the experiment was carried out by Dr. Petersen at the Institute for Research into Animal Nutrition at Brunswick, while Prof. Seher in Münster was responsible for the analysis of tissue lipids.

The histometric examination of the hearts required a sampling method suited to the volume of the heart in pigs. In order to do this we cut the cardiac muscle up into seven regular slices with the aid of a restraining apparatus specially constructed for this purpose. The tissue specimens were sampled at similar pre-selected sites, for all the pigs (Fig. 10).

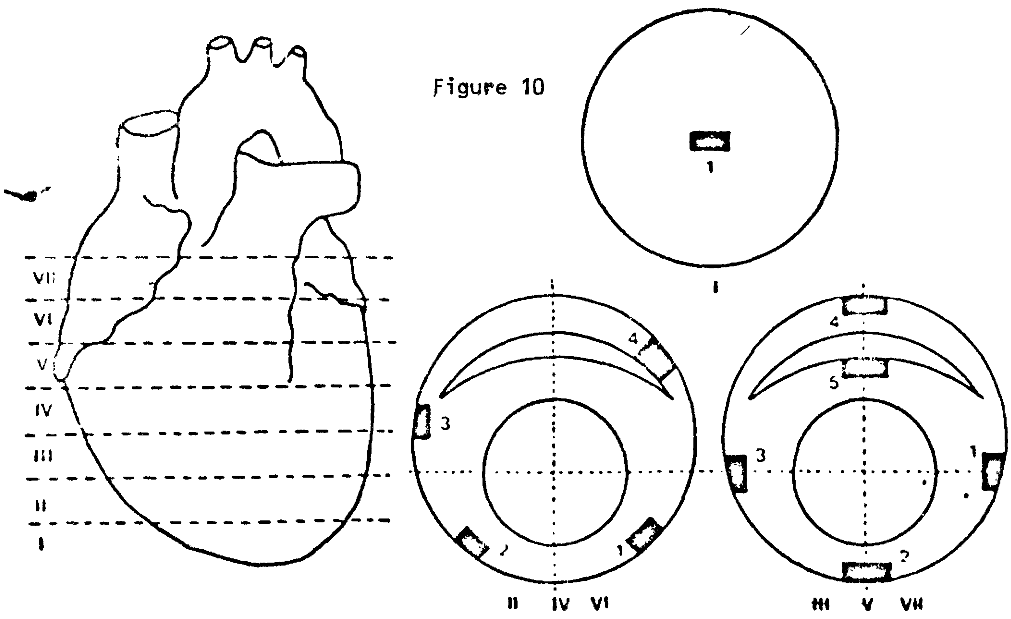

Figure 10

Thus each heart was examined at 28 different sites. All the histological sections showing anomalies were subjected to a morphometric examination identical with that carried out on rats, mice and rabbits. The lesions observed were characterized in a general way by small foci of cardiac necrosis or myolysis with leucocyte or macrophage infiltrations. The statistical analysis of all these results showed no difference between the groups. These were constituted as shown in the experimental scheme presented in Table V.

TABLE V

SCHEME OF THE 17 WEEK EXPERIMENT ON PIGS

	Control	Soya		Mixtures		Colza		Primor		Lesira
Level of incorporation	-	4	8	4	8	4	8	4	8	8
Total lipid level %	2	6	10	6	10	6	10	6	10	10
% erucic acid in diet	-	-	-	0,5	0,7	0,9	1,5	1,4	2,2	0,2
No of pigs examined	7	6	7	7	7	7	7	7	7	6

A group of animals receiving a diet based on barley meal and soya oilcakes served as controls. For the experimental groups, the lipid content was variable both qualitatively and quantitatively. They were given soya oil at two levels, mixtures of varying proportions of traditional colza oil and Primor oil (7.5, 15 and 22% erucic acid), and Lesira, a German colza oil with 1.9% erucic acid. The proportion of lipids by weight was 2, 6 and 10% of the ration, the latter level corresponding to about 25% of the total energy in the diet. Overall, there are no significant differences between the groups as regards the effect on the heart, whether assessed by the number of lesions or the sum of their Feret diameters. Figs. 11, 12 and 13 show the results of statistical analysis according to the quantity of lipids in the diet, the botanic origin of the oil, and the level of erucic acid.

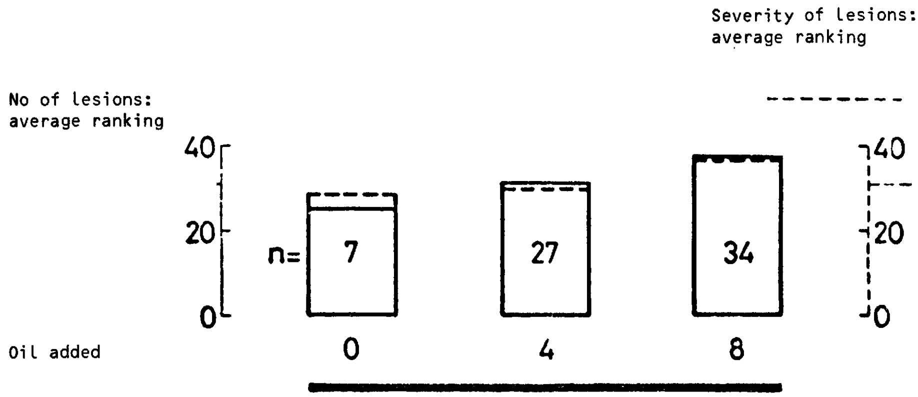

Fig. 11: Effects of diets on the myocardium in pigs

Fig. 11, which represents the ranking as a function of the quantity of lipids in the diet, shows a slight shift which however is not statistically significant.

Fig. 12 shows that Lesira oil compares very well with the other régimes.

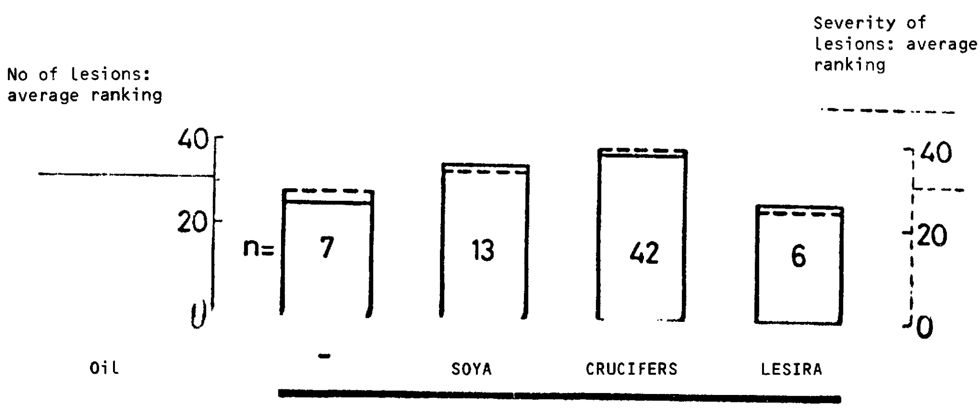

Fig. 12: Effects of diets on the myocardium in pigs.

The ranking as a function of the level of erucic acid in the diet (Fig. 13) shows that, in the case of pigs, a diet containing up to 2.2% erucic acid administered for four months affects neither the incidence nor the severity of the lesions.

The studies with mice, rabbits and pigs thus confirm the absence of negative effects which may be attributed to cruciferous oils containing little or no erucic acid.

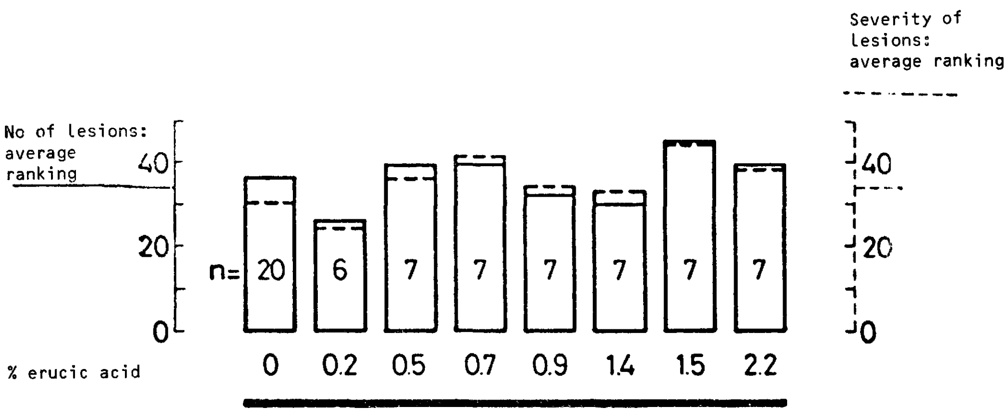

Fig. 13: Effects of diets on the myocardium in pigs

CONCLUSIONS

These results as a whole underline the step forward in nutrition represented by the development of new varieties of colza.

As regards the particular case of the Sprague-Dawley rat strain, we think we are justified in stating that the relatively high incidence of lesions observed in the long-term after the administration of considerable quantities of oil, whether Primor or linseed, can be explained by the metabolic competition between the various dietary fatty acid families represented in these particular régimes.

We have explained elsewhere the biochemical and physiological factors behind the essential role played by certain dietary fatty acids in maintaining the anatomical and functional integrity of cardiac muscle.

This background information, on which we cannot dwell here, is arousing considerable interest in specialist circles.

However, we ought to stress here that any one oil, or any one food product, is rarely ideal or universally applicable. Nor can one really say that there are bad or good foodstuffs, but on the other hand bad nutrition does exist.

It is therefore important in studying nutritional phenomena to consider the balance, taking into account both quantitative and qualitative aspects of the components of the diet.

Further experimental data are still essential for enlarging our knowledge. Their use in practical everyday nutrition, which is generally varied and heterogeneous, will take into consideration the ways in which the product being studied is used. One should not lose sight of the fact that dietary lipids include a mixture of visible and invisible fats and oils which may either correct or aggravate the effect of a partial imbalance.

For the sake of completeness we feel we should point out that the botanical designation of an alimentary oil gives only a partial picture of its fatty acid composition. This may vary according to such factors as variety, growing conditions or the way it is processed.

Thus, in the choice of the approach to be adopted, the nutrition expert has to guide both the agronomist and the technologist, in the consumer's interest. The identification of antinutritional factors and the clarification of the way they work enable the world's resources to be used more wisely, and ours is a world where plenty and privation exist side by side. Thus, to outline an improvement in nutrition is to make some small contribution to the world's well-being.

SYMPOSIUM ON NEW VARIETIES OF COLZA

CONCLUSIONS TO BE DRAWN FROM THE SYMPOSIUM

by Mr André François

Director of the Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation (C.N.E.R.N.A.)

RAPPORTEUR

12 April 1978

Mr Chairman,

Ladies and Gentlemen,

As I listened to the comprehensive and interesting papers which have been given during this symposium, I thought that it was unwise of me to have agreed to summarise all this research work and to draw conclusions so quickly. I will not attempt to be exhaustive, but merely sketch the main lines of the ideas arising from the experimental results which have been described to us.

My first reflection will be a general one on research. You know that we often speak "pure" research, which has no immediate social or economic purpose. This concerns many aspects of mathematics, physics, chemistry or biology which provide us with vital theoretical bases. Then there is a second type of research, perhaps better called "directed" rather than "applied". This type of research has a strong social and economic impact because it is oriented towards applications which concern our everyday life. Nutrition and food are part of this. About 30% of people's household budget is spent on food. What is more, there are many links between food and health and this often has major consequences; we have had examples of this in particular in the case of heart disease.

Of course nutrition experts are also concerned with pure science; there must be a balance between pure science and application. The only nutritionist who remained a fundamentalist died without knowing that he had been a nutritionist. This was Lavoisier, the man who initiated the science of nutrition, because he showed life to be a combustion process and thus introduced the concept of a quantitative balance sheet.

In the case with which we are concerned, that is, the nutritional value of oils and in particular crucifer oils, there are three aspects: fundamental research involving for example the study of mechanisms of metabolic competition between fatty acids; I should mention that in my opinion this is a line to be followed in the future. The second aspect is that of the application of scientific knowledge to feeding. This involves recommendations on diet: what should we eat? What are the nutritional objectives? The third aspect covers recommendations concerning the production of human food and consequently agricultural production: what should be produced in order to comply with the diet recommendations? Here we come up against a major problem of food policy, that is, guiding production on the basis of health requirements. In any case, I think that research is always beneficial. I like to think this is shown by the case with which we are concerned today. At the start, the research field was essentially colza oil; then, once under way, progress was made on the elimination of glucosinolates. So this research has had much beneficial spin-off.

Research can be an aim in itself; one can spend thirty years, for example, studying the sexual deviations of the female mosquito in Amazonia. One could also work for 30 years on colza and discover facts of great scientific interest. However, scientific policy has its requirements. Admittedly, nothing is ever complete and final in science. It is always possible to discover something new, this is what knowledge means, knowledge is ever total. But the moment arrives when a reasonable level of knowledge has been reached. This level has to be assessed by the international scientific community on the basis of the facts contained in the research reports, but also in the light of budgetary constraints. Research is dear and is a burden on taxpayers, that is, on consumers. A reasonable balance has to be found between the research programmes to be carried out and the importance of the problems to be solved.

It seems to me that we have now reached the critical mass of knowledge needed to give us a clear idea of the nutritional value of colza oil. Now we have to translate this scientific knowledge into clear simple recommendations for consumers and for the competent public authorities. This is perhaps the most difficult stage, because for researchers the truth is never completely black or white. Therefore many hesitate to commit themselves to practical recommendations.

We should not forget that millions of units of account have been spent. If it were necessary, the research ought to be continued, but as I have been following this research from the beginning I can say quite objectively that sufficient progress has been made, in particular in the last two or three years. A clearer ranking of problems has emerged. In the past it could be said that one could not see the wood for some of the trees. The male albino rat to some extent obscured what happened in other species. As regards technologies or techniques, it would have been desirable to turn earlier to more sophisticated but also more reliable histological procedures. These are some points which seem genuinely constructive and decisive to me.

So what are the main points on which we can base our findings? As regards production, colza is a plant which has traditionally been cultivated and consumed for a long time in many regions, a plant which is useful because it is high-yielding and relatively easy to grow. It is a dual-purpose plant which produces oil and high quality protein. Indeed, it is quite likely that in the future a large proportion of protein supplies will come from colza.

Of course, not everything about colza is perfect: we have heard about the adverse effects of glucosinolates and erucic acid, and also of the relatively high qualities linolenic acid and the shortage of palmitic acid. But this can be corrected sooner or later by breeding work.

Moreover, the variety of fatty acids consumed by man enables a good nutritional balance to be achieved.

I shall not return to these aspects but in passing I should like to emphasize once again the enormous progress that has been made, that now we can guide food production in line with nutritional objectives. This seems to me to be a most important change in thinking, both for health authorities and at the same time for consumers.

As regards colza oil, the file contains studies both on animals and on man. I should say that when over 10 years ago we said that research should be undertaken on this subject, we came up against some scepticism. Medical circles said that we were investigating a murder where there was no body. What's the problem, they asked? There is no specific human pathology, what do you want to research? I replied then that although there was as yet no colza pathology, there might be one if the consumption of colza increased. In experimental toxicology one must have experimental models. The lesions observed in male albino rats were rather worrying and it was worth while trying to have a clearer view by taking experimentation further and by extending its scope.

This explains why there was a vigorous development of research work over a certain number of years and I should say again that in the last two or three years this has been organized along more coherent lines. The main aim was comparison of different oils, whether colza or other current oils used in food. Researchers made comparisons between the effects observed on various animal species; they studied the influence of sex. Improvements were made in histological techniques, in particular by quantitative histometry. Large-surface sections were made, in order to improve the way in which the morphology of cardiac tissue could be investigated. Blind comparative tests were made without questioning the objectivity of histopathologists. The effect of increasing doses of oil were also studied.

This is why concepts have been able to evolve over the last few years. What are the main conclusions to be drawn from the papers which we have heard?

As regards the role of erucic acid I think that it is pointless to come back to this. Its cardio-toxicity has been demonstrated, as also has its adverse effect on the components of the blood in particular since it encouraged a platelet aggregation and coagulation of the blood. This is a further important reason for eliminating erucic acid. It was this that led to the regulations.

As regards crucifer oils free of erucic acid, do they cause death in test animals? Little work has been published on this subject. It has been reported here that animals which have received these oils continue to live for the same length of time. Mortality is not increased by conditions of stress through cold.

It is however true that these oils can cause lesions in rats; however, they occur at a later stage, are less serious, less numerous and only appear at the highest ingestion rates. On the other hand, histopathological examinations on organs other than the heart, such as kidneys, liver, etc, have not revealed any abnormalities. Toxicity tests have been carried out on hens. This is a very sensitive test. When there is a toxic substance in the feed the hatchability rate of the eggs may be affected. This is not the case for oils without erucic acids.

Is the factor still causing this lesser degree of later, less serious damage to the myocardium in rats some factor in the unsaponifiable fraction or a contaminating factor in the glycerides? A large number of experiments have been described: fractionation, purification of the glycerides, concentration of the unsaponifiable fraction. The answer is no: there is no factor X which produces the cardiac lesions observed when crucifer oils are administered. Finally, we heard about a whole series of comparative tests on oils: groundnut, sunflower, soya, palm, colza without erucic acid. Tests have been carried out on rabbits, rats and pigs and the essential conclusion to be drawn is that there is no effect which is specific to crucifer oils and particularly to colza oils with a low erucic acid content. There was a residual pathology in all cases and according to statistical analysis this residual pathology is greater the older the animals are. To what is this due? Is there an imbalance between the fatty acids? We have heard information indicating that this would be an interesting avenue to explore: for example, can excess linolenic acid block the synthesis of linoleic acid? This seems a very interesting possibility.

Let us now examine not the oil but the factors connected with animal species. The speakers we have heard have compared the reactivity of species. They have found that there were no lesions in pigs and monkeys, and they have shown up the particular sensitivity of male albino rats, as used in many tests at the start of research into colza; certain strains of rats may even be particularly sensitive. However, when there is 5 or 10% in their food, there is no difference between oils, whereas at over 15% of primor for example one begins to become apparent. The dose therefore has some effect. We were also reminded that dilution caused the effects observed to disappear, even with particularly sensitive animal species.

We have thus observed in animals a number of facts which seem to me to be coherent. What consequences can one draw from this for human nutrition? As I have pointed out, we do not know of any human pathology for colza, but this does not mean that it could not exist in particular circumstances. On the other hand, heart diseases linked with nutritional errors occur frequently and have very serious consequences for men. Women are perhaps less sensitive, but from 40 onwards almost one man in two is subject to an increased risk. This is a really serious problem. Unsaturated fatty acids are if anything beneficial in the prevention of thrombosis and varieties of oil which are low in erucic acid, far from increasing the risk, tend to decrease it.

Such is the brief outline, perhaps too rapidly drawn, of what the wide-ranging papers we have heard have brought us. These, at any rate, seem to me to be the most interesting facts. What conclusion is to be drawn?

In my opening address I stressed the responsibilities of the research worker and some questions on this subject have been raised during discussions. It is a question of drawing practical conclusions from research and I have some responsibility in my country for guiding research. Obviously, one must know what are the most important problems. As things stand at present it is clear that cardio-vascular diseases have priority over other problems such as any possible pathology of colza oil. From what we have heard we can say that the probability of the existence of such a pathology is slight.

I therefore believe that the comparative tests which have been described allow us to say that colza oil of the new low erucic varieties can resume their place among the other oils.

Until the last few years, for reasons of which you are aware and which were perfectly justified until sufficient information was available, colza has been blacklisted, as it were. It is obvious that the results which have been described here, together with those published previously, show that there is now a group of food oils, from which none can be excluded. There should no longer be any <u>a priori</u> segregation based on nutritional characteristics. As in all families there are some members which have particular characteristics; some oils can be heated; some have beneficial effects concerning disease, but what is available to consumers is a homogeneous group of products. It is the consumers who must choose the type of oil which they prefer. The problem of competition between the various oils and fats, butter, margarine, etc., has been somewhat left behind, and in the future we shall have to talk in terms of fatty acids.

Consequently information plays a large role. A Symposium such as this, in spite of the difficulties in the high level of the papers for those who are not familiar with this kind of problem, is shown to be thoroughly beneficial.

Our doctor colleagues have insisted that, if any recommendation is to be made, then it must concern the total consumption of oils and fats. This is to be limited in view of the links between excessive calorie intake and cardio-vascular disease in particular.

This information meeting seems to me to have been extremely useful and I should like to thank and congratulate the Commission and in particular Mr Waechter for having organized it. I said in opening the discussion that the case of colza seemed to me exemplary from many points of view. If this is the first information meeting which you have arranged, Mr Chairman, I certainly think that it will not be the last, for I consider it would be a good idea for gatherings of the same kind to be organized on other food products.

COMMISSION OF THE EUROPEAN COMMUNITIES

SYMPOSIUM
ON
NEW VARIETIES OF COLZA

DEFINITION OF CERTAIN CHEMICAL TERMS USED
in the field of fats and oils

- NATURAL FATS -

These consist chiefly of triesters of glycerol and fatty acids known by the name of triglycerides.

They also contain, always in small quantities, other components, known collectively as minor constituents, in particular :

- phospholipids,
- sterols,
- fatty alcohols,
- tocopherols,
- pigments,
- hydrocarbons,

and traces of free fatty acids.

TRIGLYCERIDES

- FATTY ACID TRIESTERS -

These are the substances formed by the combination of three fatty molecules with one glycerol molecule.

- GLUCEROL, commonly called "GLYCERINE" -

This is the simplest of the trihydroxy alcohols.

- FATTY ACIDS -

These are molecules consisting of an aliphatic chain and a weak function (carboxyl group).

Natural fats usually consist of the triglycerides of four to eight different fatty acids.

Depending on their chemical structure, fatty acids are divided into several categories :

- short-chain acids
- medium - and long-chain acids
- very-long-chain acids
- saturated acids
- unsaturated acids.

a) The classification based on the length of the aliphatic chain distinguishes between fatty acids according to the total number of carbon atoms they contain :

- short-chain, up to 10
- mediup-chain, between 12 and 14
- long-chain, between 16 and 18
- very-long-chain, 20 and over.

In everyday language, however, the term "short-chain fatty acids" generally denotes all fatty acids with fewer than 14 carbon atoms, while "long-chain fatty acids" denotes those with at least 20 carbon atoms. Acids containing 14, 16 or 18 carbon atoms are called simply "fatty acids".

b) The distinction between saturated and unsaturated fatty acids is based on the chemical structure of the aliphatic chain and, consequently, on its chemical properties.

- Saturated acids are those in which all the carbon atoms of the aliphatic chain are linked by single bonds.
  Owing to the stability of this type of bond the aliphatic chain of these acids is very stable, especially in regard to oxidation (i.e. they do not become rancid).

- Unsaturated acids are those in which at least two carbon atoms of the aliphatic chain are linked not by a single bond but by a double bond (ethylenic bond).
  The relative instability of this type of bond renders the aliphatic chain less stable and allows it to react with many substances, e.g. :

  - atmospheric oxygen, which gives rise to oxidation products responsible for rancidity;
  - hydrogen, which transforms unstable unsaturated aliphatic chains into stable saturated aliphatic chains;
  - iodine, which is used in the laboratory for measuring the degree of unsaturation of aliphatic chains as expressed by the iodine index, the value of which increases with the degree of unsaturation.

  Among the unsaturated acids the following are to be distinguished :

  - mono-unsaturated (mono-ethylenic) acids or mono-enes, which contain only one double bond in their aliphatic chain;
  - poly-unsaturated acids, which contain at least two double bonds in their aliphatic chain, especially di-ethylenic acids or di-enes and tri-ethylenic acids or tri-enes.

Short-chain saturated acids are liquid at ambient temperature.

Unsaturated acids, irrespective of the length of their aliphatic chain, are generally liquid at room temperature. For equal chain length, they are the more fluid the more double bonds they contain.
Certain unsaturated acids, however, are solid at ambient temperature : the double bond in an aliphatic chain can exist in two different forms : the cis form, which is the more common in natural products, or the trans form, which is rarer and gives the unsaturated acid molecule a geometrical structure not unlike to that of the saturated acids and thus causes the physical properties and some of the chemical properties to resemble those of saturated acids.

Long- or very-long-chain saturated acids are solid at ambient temperature.

The saturated acids most frequently encountered in fats are in practice denoted by names which to some extent reflect their origin.

Saturated acids

Number of carbon atoms in the molecule	Simplified designation	Common Name
4	C4	Butyric acid
6	C6	Caproic acid
8	C8	Caprylic acid
10	C10	Capric acid
12	C12	Lauric acid
14	C14	Myristic acid
16	C16	Palmitic acid
18	C18	Stearic acid
20	C20	Arachidic acid
22	C22	Behenic acid
24	C24	Lignoceric acid
26	C26	Cerotic acid

Mono-ethylenic acids

The most frequently encountered mono-ethylenic fatty acids with 12 to 22 carbon atoms are denoted in practice by names derived from the corresponding saturated acid.

For one and the same mono-ethylenic acid, however, there are several isomers which differ from each other : either by the position of the double bond in the molecule (the carbon atoms are numbered in sequence, starting at the acid groupe °, and the position of the double bond is indicated by the number of the first carbon atom participating in it), or by the form of the double bond (cis or trans).

Number of carbon atoms in the molecule and form of the double bond	Simplified designation	Position of the double bond	Name
12 cis	C12	9	Lauroleic acid
14 cis	C14	9	Myristoleic acid
16 cis	C16	9	Palmitoleic acid
18 cis	C18	9	Oleic acid
18 trans	C18 t	9	Elaidic acid
18 cis	C18	6	Petroselinic acid
20 cis	C20	11	Gadoleic acid
22 cis	C22	11	Erucic acid
22 trans	C22 t	11	Brassidic acid

° In biochemistry a different nomenclature is used to specify the position of the double bond in the molecule.

Poly-ethylenic acids

The best known and the most important of the poly-ethylenic acids occuring in fats are the C_{18} acids.

Number of C atoms in the molecule	Number and form of double	Simplified designation	Position of double bonds	Name
18	2 C-C	18''	9-12	Linoleic acid
18	3 C-C-C	18'''	9-12-15	Linoleic acid
20	4 C-C-C-C	C_{20}''''	5-8-11-14	Arachidonic acid
22	5	C_{22}'''''	4-8-12-15 -19	Clupanodonic acid

PRINCIPAL MINOR CONSTITUENTS

A - PHOSPHOLIPIDS -

These are triglycerides in which a fatty acid molecule is replaced by a phosphoric acid molecule generally bound to nitrogenous bases.

The best known is lecithin, which not only occurs in fats but is also one of the main constituents of eggs.

B - STEROLS -

The various sterols present in fats are cholesterol derivatives in which one or both of the hydrogen atoms attached to carbon atom 24 have been replaced by groups with varying degrees of unsaturation. The structure of the principal sterols is as follows :

Name	Empirical formula	Substitution at position 24	Number of double bonds	Position of double bonds
Cholesterol	$C_{27}H_{46}O$	-	1	5
Ergosterol	$C_{28}H_{44}O$	CH_3	3	5-7-22
Brassicasterol	$C_{28}H_{40}O$	CH_3	2	5-22
Campesterol	$C_{28}H_{48}O$	CH_3	1	5
Stigmasterol	$C_{29}H_{48}O$	C_2H_5	2	5-22
β-Sitosterol	$C_{29}H_{50}O$	C_2H_5	1	5
-Stigmastenol	$C_{29}H_{50}O$	C_2H_5	1	7

Cholesterol is the chief component of all animal fats; it is present only in small quantities in the sterols of vegetable fats, except those of palm oil, in which it may constitute 8% of the total sterols.

C- TOCOPHEROLS -

These are natural anti-oxidants which occur only in vegetable fats. The most common is α- tocopherol, a complex cyclic alcohol with 29 carbon atoms, otherwise known as vitamin E.
The principal other tocopherols bound in vegetable oils are β -and γ -tocopherol with 28 carbon atoms and δ -tocopherol with 27 carbon atoms, which have no vitamin properties and which are anti-oxidants, i.e. substances that prevent oils from turning rancid.