Nutritional Effects of Hydrogenated Soya Oil

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ABSTRACT

Soybean oil is the leading edible vegetable oil in the world in terms of volume, and considerable amounts are consumed in partially hydrogenated forms. Early recognition that commercial hydrogenation of vegetable fats produces isomeric forms of monoenes and polyunsaturates has prompted much research and speculation on the nutritional properties of hydrogenated fats, including soya oil. Past results of studies with animals and humans will be reviewed and key findings will be summarized and discussed. Particular attention will be devoted to questions recently raised concerning the relationships of health and hydrogenated fats and an attempt will be made to put these matters into factual perspective in light of current knowledge.

Soya oil (soybean oil = SBO) is the leading edible vegetable oil in the world in terms of volume. USDA data (1) for 1978/79 world production of edible vegetable fats and oils showed (in 10^6 metric tons [MT]): soybean, 11.9/37.6 = 32%; sunflower seed, 4.7/37.6 = 12.5%; palm, 4.1/37.6 = 10.9%; rapeseed, 3.7/37.6 = 9.8%; peanut, 3.4/37.6 = 9.0%; cottonseed, 3.0/37.6 = 8%; and all others, 6.8/37.6 = 18%. The U.S. is a leading producer and consumer of SBO. Preliminary USDA data (2) suggested U.S. 1979 production was 5.5×10^6 MT and consumption was 4.2×10^6 MT. Dutton (3) has pointed out that ca. 60% of the U.S. edible fats and oils are partially hydrogenated and Emken (4) indicated that partially hydrogenated soybean oil (PHSBO) made up 43.9% (ca. 30 g/capita/day) of the U.S. total visible fat consumption in 1976.

Hydrogenation is an important unit process in the production of edible products from SBO. It is used to improve melting points and plasticity of base stocks for shortening and margarines and to improve the flavor and odor stability of products using PHSBO. There was very early recognition that much of the functional improvement in PHSBO was related not only to saturation during hydrogenation, but to isomerization of *cis* double bonds to the *trans* monoene configuration. Included is the elimination of linolenic acid (improving flavor stability) and, depending on hydrogenation conditions, some linoleic acid is hydrogenated to a mixture of *cis* and *trans* monoenes and various *cis-cis*, *cis-trans* and *trans-cis* isomers. Typical compositional data (5) are shown in Table I.

The changes in fatty acid composition just outlined have been of interest to biochemists, nutritionists and health professionals for many years. An indication of current interest can be found in the large number of meeting programs, monographs (e.g., ref. 3), research grant applications and publications devoted wholly or in part to the nutritional and metabolic effects of hydrogenated fats. This review aims to summarize and discuss past and current germane work and attempts to bring some perspective to the recent questions raised regarding the relationships of health and partially hydrogenated fats.

Before we go further, we should recognize the complexity of the isomer mixtures formed during commercial, partial hydrogenation. As reviewed (3), there are a number of *cis* monoenes formed. These are mainly the *cis*-9 and *cis*-12 monoene isomers with only small amounts in the 8-, 10-, 11- and 13-positions (Fig. 1). The *trans* monoene fractions, however, appear to be largely *trans*-10 and *trans*-11 with lesser amounts at various positions out to the 6th and 14th carbons (Fig. 2). As noted in Table I, the number of *cis-trans* nonconjugated dienes is quite limited and the *trans*-9, *trans*-12 isomer was not found. We will consider the nutritional relationships of these points later, but it is important to recognize that the dienes are minor components in PHSBO.

Emken (4,6) recently reviewed the literature on the absorption and transport of fats. With the exception of fully saturated triglycerides, such as tristearin, which are absorbed poorly, the partially hydrogenated fats have digestibilities in the 79-98% range. Some of the classical work conducted on partially hydrogenated fats is that of Alfin-Slater and coworkers (7). Alfin-Slater and Aftergood recently reviewed this work and other studies, as well (7,8). In long-term multigeneration studies (over 30 years) with rats fed a margarine fat containing 35% *trans*-fatty acids as the sole dietary fat, no problems were observed in growth (Table II), reproduction, survival, plasma and liver cholesterol levels or tissue pathology. Similar life-span studies

TABLE I

Fatty Acids of Soybean (SBO) and Partially Hydrogenated Soybean Oils (PHSBO)[a]

Fatty acid	SBO	PHSBO[b]	PHSBO[c]	PHSBO[d]
16:0	11	11	11	11
18:0	4.1	4.3	7	10.5
cis-18:1	22	29	33	18
trans-18:1	–	12	12	51
cis-9,*cis*-12-18:2	54	31	22	–
cis-9, *trans*-12 plus *trans*-9, *cis*-12-18:2	–	4	6	–
trans-9, *trans*-12-18:2	–	–	–	–
cis-9, *trans*-13 plus *trans*-8, *cis*-12-18:2	–	2	4	9
Conjugated 18:2	–	2	0.5	–
18:3, all isomers	7.5	2.3	2.0	–
Conjugated 18:3	–	0.1	–	–
C > 20	1	1	1	1

[a]Houtsmuller (5).
[b,c,d]Calculated IV ca. 110, 105 and 76.

in rats (9) and 18-month studies in mice (10) with butterfat (*trans* 4-8%), SBO, 3 PHSBO (*trans* of 8-24%, 10-30% and 53-67%) and coconut oil at 54% of calories plus 6% of calories as SBO (to meet essential fatty acid [EFA] requirements) showed no effects on life-span, tumors or histopathology that could be attributed to the PHSBO even at the highest *trans* levels. And, in earlier studies using high-fat diets (54% of calories) and high *trans* levels (54%) derived from PHSBO, no evidence of acute toxicity was obtained (11).

With these types of results, why is there an ongoing polemic regarding hydrogenated fats and health? It seems that, in part, these differences in opinion often result from real observations of events where the interpretations may have been clouded by misconceptions or a lack of complete understanding of the complexity of the system under study. Thus, seemingly unequivocal results rapidly become equivocal as other possible effects are considered.

In various articles on fats and health (12-15), several older citations on animal studies (16-20) invariably are cited as classical examples of the effect of *trans*-monoenes on serum cholesterol levels (16-18), incorporation of *trans* in tissue (19), swelling rate of mitochondria (20) and increased rate of erythrocyte hemolysis (20). These studies (16-20), however, have one common thread that makes the interpretation of the results appear equivocal. Weigensberg and his coworkers (16,17) elaidinized oleic acid (from beef tallow: *cis*-9, *cis*-12-18:2 ca. 8%) or olive oil (*cis*-9, *cis*-12-18:2 ca. 6.5%) with nitrous acid and did not purify the products. Depending on the conditions (not specified), they could have reduced the content of *cis*-9, *cis*-12-18:2 in these fats to as little as 6% of the original value if equilibrium was reached as described by Litchfield et al. (21). Further, they could have introduced as much as 56% *trans*-9,*trans*-12-18:2 and 30% mixed *cis*-9, *trans*-12 and *trans*-9, *cis*-12-isomers in place of the original *cis*-9, *cis*-12-isomer. And, the amount of nitro compounds was unknown. These possibilities were recognized (17) and exploited (18) but were not considered significant by the authors (16-18). Similar questions must be raised concerning the results reported by Decker and Mertz (19,20), for they also used elaidinized olive oil that was prepared by treatment with SO_2. Again, as clearly shown by Mattson (22), SO_2 is an effective catalyst for converting *cis*-9, *cis*-12-18:2 to a mixture of *cis, trans*-, *trans, cis*- and *trans, trans*-isomers. Assuming the equilibria observed by Litchfield et al. for Se or nitrous acid elaidinization also holds for SO_2, the amounts of diene isomers have been estimated (Table III). Thus, it appears that Decker and Mertz possibly were feeding marginal levels of EFA (cf. Table I [19], where the original 8.8% *cis*-9, *cis*-12-18:2 decreased to 0.4% with SO_2) as they acknowledged (19) and the effects ascribed to *trans*-monoenes (20) might well be related to limited EFA plus the mixed linoleic acid isomers inadvertently introduced into the diets. Also notable is that is has been shown that EFA deficiency leads to increased hemolysis of red blood cells (23).

In light of these discrepancies in such key references, it seems advisable to assess all dietary constituents introduced in controlled studies, and not just those presumed to be under study. The classical work of Anderson et al. (24) has further delineated the relationships between dietary *cis*-9,

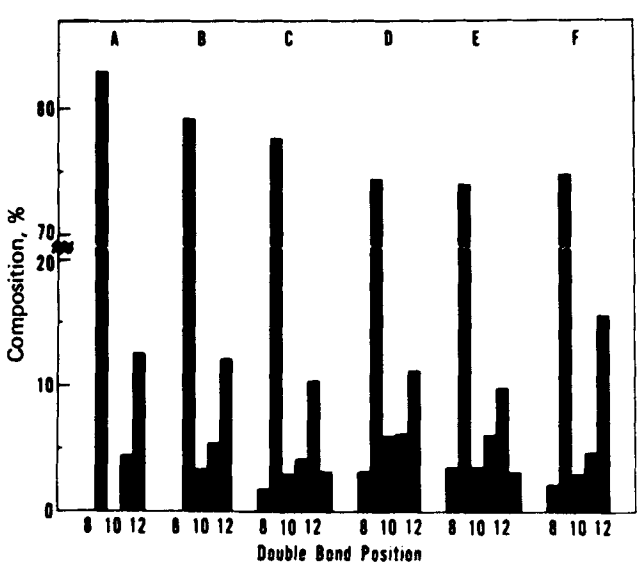

FIG. 1. Double bond distribution in the *cis*-octadecenoic fractions from 6 commercial shortening and vegetable oil samples. Scholfield et al. (1967) from (3).

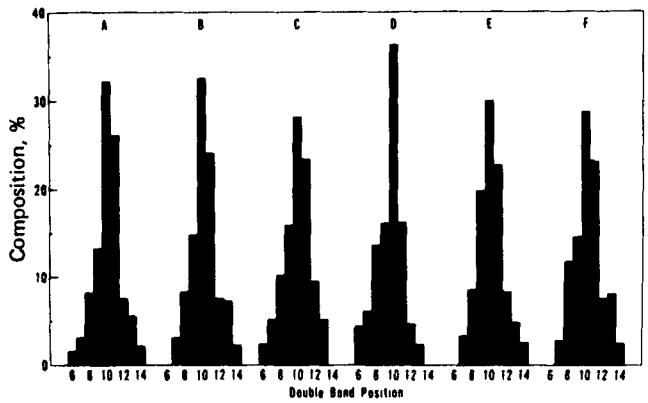

FIG. 2. Double bond distribution in the *trans*-octadecenoic fractions from 6 commercial shortening and vegetable oil samples. Scholfield et al. (1967) from (3).

TABLE II

Growth of Rats of Representative Generations in a Multigeneration Experiment, Fed Dietary Fat Containing 35% *Trans* Fatty Acids

Fat level (%)	Generation	Weight at 90 days (g)[a] Males	Females
11.2	40	290	200
	75	310	212
16.0	5	283	219
	25	278	209
Purina Chow Group		280	210

[a]Twelve males and 24 females/group, from Alfin-Slater and Aftergood (8).

TABLE III

Estimated EFA Composition: Fats Elaidinized with Nitrous Acid or SO_2[a]

	Original	Final		
		Mixed		
Fat source	*cis,cis* (%)	*trans,trans* (%)	*cis, trans* (%)	*cis,cis* (%)
Beef tallow[b]	8.0	4.5	3.0	0.5
Olive oil[c]	6.5	3.6	2.5	0.4
Olive oil[d]	8.8	4.9	3.3	0.5*

[a]Estimated per Litchfield et al. (21).
[b]Weigensberg et al. (16). Elaidinized sample was not purified.
[c]McMillan et al. (17).
[d]Decker and Mertz (19,20). *Reported (19) as 0.4%.

cis-12- and *trans*-9, *trans*-12-18:2 in rats and showed the EFA antagonism of the *trans*-9, *trans*-12-isomer. Holman's early work (25) in this area clearly demonstrated the EFA antagnoism of the *trans*-9, *trans*-12-18:2 isomer and many other groups, including Privett and coworkers (26) and Kinsella et al. (27), have published continuations of detailed studies on the metabolism of and physiological effect of *trans*-9, *trans*-12-18:2.

Much of this current interest appears to be based on misunderstandings regarding the possible presence of *trans*-9,*trans*-12-18:2 in partially hydrogenated fats (27). As already noted (5), *trans*-9, *trans*-12-18:2 has not been found in various PHSBO. Kinsella et al. (27) credit Carpenter and Slover (28) with analyses that show *trans*-isomers of linoleic are less than 5% in margarine oils, but they (27) neglected to note that, at best, Carpenter and Slover (28) found traces only of the *trans, trans*-isomer. Considerable additional evidence has been published on this topic as summarized in Table IV. In addition to the data there, it should be noted that early work in our laboratories (D.J. Roy, M. Blank and H.W. Jackson, unpublished data) on samples of margarine oils representative of those discussed as possibly containing 1.1-8.6% *cis, trans-* and *trans, trans*-isomers (35) showed at most 0.2% *trans,trans*-isomers by $AgNO_3$ adsorption chromatography and capillary GLC. Thus, as indicated by Anderson (24) and reinforced by Vles (38) and Houtsmuller (5), there should be little concern regarding the physiological effects of the *trans*-9, *trans*-12-18:2 isomer because it is not present in significant amounts in partially hydrogenated fats of commerce.

In addition to the studies already mentioned, there have been many publications, particularly in the last decade, that deal with varied aspects of the in vivo and in vitro enzymatic transformations, metabolic fate and deposition of a multitude of fatty acid isomers in various model systems with considerable attention to the *trans*-isomers. It is beyond the scope of this paper to consider all of the nuances, implied or real, that may be drawn from these model studies. Those interested should review and assess the mass of pertinent information and references recently compiled (6,12,13,39,40).

Two aspects of animal and human nutrition and health related to PHSBO that have received increased attention recently are those concerned with coronary heart disease (CHD) and other circulatory disorders and with cancer. Development of the Lipid Hypothesis in the early 1950s as recently reviewed in a provocative article by Ahrens (41), was soon followed by Sinclair's (42) suggestion that hydrogenation not only destroys EFA, but it also introduces "unnatural" *trans* fatty acids into the diet. Further, Sinclair suggested that these facts are related to, e.g., atherosclerosis and coronary thrombosis. According to Ahrens (41), the Lipid Hypothesis has not been adequately tested or proven and, in spite of many years of animal and human research, neither has Sinclair's (42).

As one point of clarification, it seems to me that the unscientific word "unnatural" has crept into our vocabularies when discussing *trans*-isomers. One might believe that only since the advent of hydrogenation some 60-70 years ago were humans exposed to such materials. Actually, humans and animals have been exposed to *trans*-isomers since they first consumed ruminant products. And, in fact, Heckers et al. (43) have estimated that 35-45% of the *trans*-fat intake of 4.5-6.4 g/capita/day in West Germany for 1976 originated in ruminant fat.

Returning to the Lipid Hypothesis and PHSBO, much of the current renewed controversy about hydrogenated fats apparently originated from the media treatment of a single study on swine feeding first reported in 1974 (44). This work showed that feeding young swine 19% dietary 65 IV PHSBO with 50% *trans* content caused the greatest ele-

TABLE IV

Trans,trans-Octadecadienoates in PHSBO

Amount (%)	Method	Reference
ND[a]	Ag^+-CCD	29
0.5	Ag^+TLC+GLC	30
ND	Ag^+-TLC+GLC	31
ND	Ag^+-TLC+GLC	32
+[b]	Ag^+-TLC+GLC	33
0.1[c]	Ag^+TLC+GLC	34
ND	NOT INDICATED	5
1.1-8.6[d]	GLC	35
0.1-3.0	GLC	36
0.55[e]	Ag^+-TLC+GLC-MS	37

[a]ND = not detected.
[b]+ = significant quantities unconfirmed.
[c]in one sample, other 4 ND.
[d]Mixture of *c,t-*, *t,c-* and *t,t-* unconfirmed.
[e]Margarine oil representative of 3% *t,t-* sample of Sahasrabudhe et al. (36).

TABLE V

University of Illinois Swine Feeding Results Summary[a]

	Plasma		Atherosclerosis grade (%)	No. of raised lesions	Dietary fat (%)	*Trans* in dietary fatty acids (%)	EFA (est'd) of cal. (%)
	Lipid (mg %)	Cholesterol (mg %)					
Beef tallow	331 ± 13	124 ± 5	5.2	1 (10)[b]	19	7[c]	3.8
Rearranged fat	342 ± 19	125 ± 8	3.8	0 (11)	19	–	16.4
Corn oil	276 ± 21	104 ± 7	5.0	2 (12)	19	–	22.1
Basal	273 ± 12	95 ± 5	6.0	3 (11)	3	–	–
Whole egg	303 ± 14	112 ± 5	4.3	1 (11)	3+	–	–
Used fat and sugar	362 ± 26	131 ± 11	8.6	3 (12)	11	20	?
trans-Fat[d]	388 ± 20	138 ± 9	10.0	7 (12)	19	50	2.9
Butterfat	332 ± 15	120 ± 7	7.3	2 (9)	19	5[c]	4.5
Cholesterol	245 ± 19	93 ± 3	5.2	2 (12)	3	–	–
Egg yolk	286 ± 13	98 ± 5	4.2	0 (12)	3+	–	–

[a]Kummerow et al. (44-48).
[b](Number of aorta observed).
[c]Heckers et al. (43).
[d]PHSBO 65 IV, <0.2% EFA.

vation of total lipid and cholesterol levels and the largest area of aortic atherosclerotic involvement of the various diets fed. Some key results on aortic involvement, total serum cholesterol, total serum lipids with *trans*-levels and dietary EFA (our estimate) are shown in Table V. Modified versions of this work were presented elsewhere (45,46) and published (47,48).

This work was questioned at two of the later presentations (45,46) and suggestions that the results should be attributed to the effect of a high fat-to-protein ratio and borderline EFA deficiency were acknowledged (47). Similar commentaries pointing out these dietary deficiencies were published in 1974 (49), 1976 (50) and 1979 (43), but neither the borderline protein nor EFA levels were acknowledged or considered by the original authors in 1978 when they published the total 1974 study (48). Subsequent feeding studies of young swine at the Universities of Wisconsin (51) and Illinois (52) using higher protein-to-fat ratios and increased EFA levels with dietary fats containing up to 45% *trans*-isomers (51) failed to substantiate the earlier results (44-48). An interesting genetic factor partially confounding such swine studies was uncovered (51) and discussed in detail (53). It was suggested that the presence of certain genetic traits controlling low density lipoproteins could be related to aortic fatty streaking in swine fed high-fat diets containing low levels of *cis*-unsaturation. Similar confounding genetic factors were mentioned by Lohman and Romack (54) in discussing their results of feeding adult swine PHSBO with 46-62% *trans*-monoene or corn oil as 50% of calories in 15 or 5% protein diets. They found statistically significantly ($P < 0.01$) greater abdominal aortic involvement (37.4% vs 33.2% and 39.8 vs 31.7%, respectively) in comparing the swine fed PHSBO and corn oil at 15 and 5% protein levels. Unfortunately, neither basal diet nor EFA levels were described (54) but, considering the type of PHSBO (with essentially zero EFA) used, it is possible that the EFA levels were borderline in the PHSBO diets unless other fat was added (cf. 44-48). Furthermore, Kummerow's group (52) has stressed that ". . . swine require a high level of linoleic acid in their diet. . . "

Review of some of the earlier literature shows that many factors have been reported as eliciting atherosclerosis in swine, including: EFA deficiency (55), 40 cal % butterfat (56), cholesterol plus fats (57), 33 cal % butterfat or egg yolk (58), magnesium and EFA deficiencies (59), cholesterol plus lard or egg yolk or lard (60,61), 13% fat:7% protein (62), 12% fat:6% protein and finally, psychosocial effects (63).

This last point is very provocative, particularly in view of the recent report (64) indicating that rabbits interacting with their keepers had more than a 60% reduction in 2% dietary cholesterol-induced atherosclerosis. In addition, this occurred without statistically significant different serum cholesterol levels, blood pressures or heart rates. Both groups (63,64) have urged attention to these psychosocial effects in designing animal studies. Nerem et al. (64) further suggested that such factors may be responsible for many of the inter- and intralaboratory anomalies and contradictions that apparently occur even when essentially the same experiment is conducted. Also conceivable is that such uncontrolled factors may relate to the extreme variability noted in many animal-based experimental results.

Summarizing the various swine studies reviewed, the evidence from the feeding trials where proper dietary management of protein, fat and EFA levels prevailed (51,52) does not suggest any unique effect of the *trans*-isomers in PHSBO on the occurrence of swine atherosclerosis. Recently, all of these points were reviewed and reinforced by Meyer (65).

Similar conclusions have been reached recently with respect to the effect of *trans*-isomers on atherosclerosis in rabbits by Gottenbos and Vles (66), Vles et al. (67) and Kritchevsky et al. (68). Also, in a recent careful reanalysis of the occurrence of *trans*-fatty acid isomers present in human myocardium, jejunum and aorta, Heckers et al. (69) found no difference in type or amount of *trans*-isomers in similar age groups dying from atherosclerosis or from non-atherosclerotic causes. Thus, they disallow the *trans*-isomer atherogenicity thesis (44-48): "Therefore Kummerow's observation that *trans*-acids are probably more atherogenic than others, based on feeding experiments in swine, cannot be supported."

As noted by Hornstra (70) arterial thrombosis also is a major factor in coronary heart disease. He has reviewed the role of dietary fats in this event and described some elegant work on various fats (70-73). The key results are shown in Figure 3. Summarizing this work, Hornstra notes that linoleic (*cis*-9, *cis*-12-18:2) and possibly linolenic (*cis*-9, *cis*-12, *cis*-15-18:3) acids are antithrombogenic and oleic acid (*cis*-9-18:1) is neutral. Dietary elaidic acid (*trans*-9-18:1) in a 1:1 mixture with oleic (*cis*-9-18:1) induced a nonsignificant difference in "obstruction time" compared to oleic acid alone and referring to these results (Fig. 3, points 7 and 8), he notes that this accounts for the close agreement between the effects from olive oil (71% *cis*-9-18:1) and PHSBO (58% *trans*-9-18:1). This PHSBO was "supplemented with some linoleic acid in order to prevent EFA deficiency" (70).

In some corollary blood platelet aggregation studies in Finnish men (71), it was shown that higher levels of dietary linoleic acid (12 vs 4 cal %) and P/S ratios (1.6 vs 0.25) were reflected in longer aggregation times and much less platelet aggregation. Studies of other fatty acids were not mentioned, nor were any conclusions drawn other than the guarded suggestion that the lessened aggregation associated with the group consuming the 12 cal % linoleic diet was related to the significantly reduced CHD mortality in that group observed over a 12-year period.

Emken (4,6) has included a review of the key literature on past "controlled" human studies of cardiovascular disease as related to hydrogenated fats, including PHSBO. Although considerable space (ref. 6, pp. 110-118) was devoted to this discussion, the summary (Table V of ref. 6) which admittedly is "oversimplified" only tells part of the story as noted (6). The fact that there also is a question about the existence of a relationship between serum cholesterol levels and CHD tends to confuse matters. Reiser (74) recently reviewed this situation and suggested we pos-

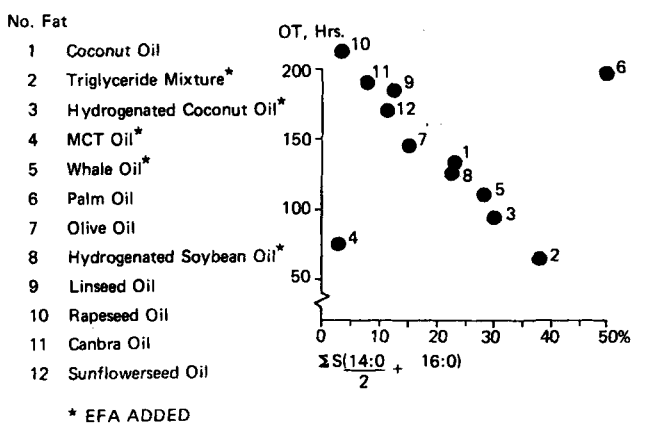

FIG. 3. Relationship between saturated fatty acid content (Σ S) and thrombogenicity (obstruction time) of dietary fats from Hornstra (70).

sibly could resolve that controversy if we could agree to the level above which serum cholesterol becomes a risk factor for CHD, if we could make a reasonable estimate as to the number of myocardial infarctions attributable to hypercholesterolemia and if we knew the number of such incidents we could avoid by lowering serum cholesterol via dietary means.

Even if we could agree to all these requirements for resolving the questions, are we confident that the changes in serum cholesterol tabulated (4,6) for human studies are results of *trans*-isomer level, dietary factors or some other variable? To illustrate these points, the original table (4,6) has been modified as shown here (Table VI). Subjects, estimated percentage of dietary *trans* fatty acids, mg % change in serum cholesterol, estimated level of EFA and presence or absence of dietary cholesterol are indicated. These data and the following discussion were developed from a detailed review of the original papers. Where unavailable, reasonable estimates of various dietary components were made based on experience with similar hydrogenated fats.

The first authors (75) made no comment regarding the effect of *trans*-isomers on serum cholesterol but seemed to regard their results as an EFA effect which appears likely from the >30-fold decrease (cf. footnote b, Table VI and p. 525, ref. 75). The next group of authors (76) made no suggestions other than those related to the reduced level of EFA in hydrogenated fats. Again the overwhelming effect of very large changes in *cis*-9, *cis*-12-18:2 should not be overlooked. Horlick (77) interpreted his results with two margarines and corn oil (Table VI) relative to control with unspecified fat type as meaning that the *trans*-isomers in the margarines were responsible for the difference between the serum cholesterol levels of the margarines and corn oil. He noted, however, that shifting dietary fat to butterfat (normal linoleic is ca. 3%) caused an *immediate* increase in serum cholesterol to "control" levels of those on margarine diets. Rather than a "*trans*-effect" (77), it is much more tempting to base these serum cholesterol results on the gradation in experimental linoleic levels ranging from 56% down to 3%. Further to this point, the serum cholesterol levels from feeding the two margarines did stabilize (Table VI) at ca. 10% below the control which mitigates against a "*trans*-effect" of any consequence.

Anderson et al. (78) felt their first three sets of results did not clearly define effects of *trans*-isomers but that their final experiment (listed in Table VI) did. Their conclusion was based on assumed "equivalency" of dietary fatty acid type and amount with exception of the *trans*-isomers. This is questionable, for the EFA ratio in the two diets was 1.8:1 and not 1:1, as they assumed by including all of the *trans*-containing dienes (>12% nonconjugated and conjugated) as part of the experimental EFA. Thus, it is again likely that these authors measured an "EFA-effect" complicated by diene-isomers and attributed it to a "*trans*-effect." Mattson and coworkers (79) also offered a similar interpretation of the Anderson et al. (78) results, noting that the experimental diet contained 10% of the total fatty acids as *trans, trans*-nonconjugated and conjugated dienes.

In the studies reported by Antonis and Bersohn (80,81), no comment was made regarding the effect of hydrogenation other than on the reduction of EFA. With such a large decrease (32-fold) in EFA, it seems likely that the elevation in serum cholesterol was related strongly to this factor. Further, though not shown here (Table VI), these authors (81) found even greater elevations of serum cholesterol with butterfat (containing less EFA) at the same level. The latter point is clouded, however, for some additional dietary cholesterol undoubtedly was introduced with the butter.

Beveridge and Connell (82) evaluated eight margarines with *trans*-contents ranging from 12-48% and GLC *cis*-18:2 contents of 7.2-27.5%. There was no apparent compositional relationship between these sets of values, however. And, as the authors noted, the three margarines that led to statistically significant increases in serum cholesterol over that of the fat-free control period could not be correlated as to their composition. That is, the *trans*-levels (35, 34 and 31%, respectively) were not the highest nor lowest and neither were all the *cis*-18:2 (7.2, 8.5 and 14.8%, respectively) although two of the latter were among the three lowest *cis*-18:2 samples. Interestingly, the sample with highest *trans* (47.8%) and 19.1% *cis*-18:2 resulted in a very low and nonsignificant (3.6 mg %) increase in serum cholesterol. Thus, any conclusion that the results noted were related to a "*trans*-effect" seems unwarranted.

In an elegant feeding study using two PHSBO and two PH cotton-soy blends together with a cottonseed oil control, an animal fat and butterfat, McOsker et al. (83) clearly demonstrated no serum cholesterol elevation with any of the partially hydrogenated fats where the *trans*-monoenes ranged from 8.5 to 17.2% and the *cis, trans*-nonconjugated dienes from 3.4-8.0%. Experimental fats had *cis*-9, *cis*-12-18:2 of 9 to 29%. The authors stressed the careful dietary control enforced including the consumption of the entire dietary amounts and isolation of the prisoners to prohibit the introduction of other food sources.

In a companion study, Erickson et al. (84) elaborated further on the previous studies using formula diets containing fats with nearly equivalent saturate, EFA and monoene levels with and without cholesterol. Nearly 11% *trans*-isomers were introduced with PHSBO. The control fat was an olive oil, safflower oil and cocoa butter blend differing only in *trans*-isomer content. Additional diets without cholesterol and with varying EFA/saturate levels showed no effects of *trans* or P/S ratio on serum cholesterol levels. The key point is that no serum cholesterol elevation was detected that could be attributed to fatty acid composition. Again, rigorous dietary control was exercised over the subjects.

Another well-controlled study by deIongh et al. (85) demonstrated the striking effect of EFA on serum cholesterol and could not substantiate the Anderson et al. (78) suggestion that *trans* has an effect comparable to saturates. All of the results observed agreed with their idea (85) that the effect of *trans* must be very small. Note in Table VI (entries 3 and 4, ref. 85) that adding only sufficient liquid soybean and sunflower oils to the 70% *trans* PHSBO to provide EFA requirements completely eliminates any significant increase in serum cholesterol (168 vs 159 mg % for the control).

Although Emken (4,6) chose to include the results of Mishkal and Spritz (86) in his summary table (ref. 99 in this ref. 6), these data will not be included here for reasons discussed earlier. These authors used trilinolein isomerized with SO_2 to high levels of *trans*-9, *trans*-12-18:2 and, as noted before, such fats have little, if any, relationship to the composition of the isomers found in PHSBO.

Mattson and coworkers (79) fed prisoners fat with 34% *trans* monoene levels, 9% *cis, trans*- and 1% *trans, trans*-isomers in formula diets containing cholesterol. As before (83,84), very rigid dietary control was exercised. The results clearly show that, in these studies, there was no effect of *trans* on either plasma cholesterol or triglyceride levels.

Considerable significance regarding the *trans*-effect has been attributed (4,6,12,13,44,87) to the last four entries in Table VI that originated in interesting work reported by Vergroeson (88) and elaborated on with Gottenbos (89). These authors (89) noted, however, "The course of experi-

TABLE VI

Variables in Studies on *trans* Fats and Human Serum Cholesterol Levels

Hydrogenated fat source (IV)	Subjects (no.)	Estimated % *trans* fatty acids	Estimated % EFA control/exp.	Presence of dietary cholesterol (±)	Change in Serum Cholesterol (approx. mg %)	Reference
Peanut (55)	Bantus (3)	44[a]	15/<0.5[a]	–	+ 30[b]	75
Corn (80)	Patient (1)[c]	26	56/6	–	+ 73	76
(58)	(1)[c]	32	56/0	–	+ 98[d]	76
(80)	(1)[e]	26	56/6	–	NC[f]	76
Cottonseed (68)	(1)[g]	32	48/5	–	+ 40[f]	76
Margarine (95)	Students	?	?/28	–	- 20[h]	77
Margarine (88)		?	?/9	–	- 20[h]	77
Corn oil (liq.)		?	?/56	–	- 50[h]	77
Safflower (~ 70)	Patients (27)	33-37	78/13	+	+ 10[i]	78
Safflower (~ 70)	(12)	33-37	78/13	+	+ 25[j]	78
Corn (~ 70)	(13)	37	57/6	+	+ 21[j]	78
Corn (~ 70)	(23)	34	24/14[k]	+	+ 21[l]	78
Sunflower (~ 70)	Prisoners (30)	48	64/2	+	NC[m]	80
Sunflower (~ 70)	Prisoners (24)	48	64/2	+	+ 28-36[n]	81
8 Margarines (68-96)	Students (8-10)	12-39	3/4.2-14.7	–	NC[o]	82
8 Margarines (77-95)	Students (7-9)	21-48	3/4.1-15.3	–	(5) NC[p] (3)+ 12-16[p]	82
Soybean (109)	Prisoners (42)	15	56/29	–	NC[q]	83
Soybean (100)	(42)	18	56/22	–	NC[q]	83
Cotton-soy (95)	(42)	14	56/23	–	NC[q]	83
Cotton-soy (76)	(42)	21	56/9	–	NC[q]	83
Soybean (108.5)	(42)	10.6	35/31	+	NC[r]	84
Soybean (108.5)	(42)	10.6	35/31	–	NC[r]	84
Whale Oil	Patients (72)	50	61/0	+	+ 49[s]	85
Soybean	(72)	10	61/37	+	NC[s]	85
Soybean	(72)	70	61/0	+	+ 35[s]	85
Soy-sunflower[t]	(72)	50	61/11	+	NC[s]	85
Soy-safflower	Prisoners (30)	44	16/9	+	NC[u]	79
Coconut-olive-safflower	Monks and nuns (23)	34	34/34	+	+ 12-13 (7-8)[v]	88(89)
Coconut-olive-safflower	(24)	34	10/10	+	+ 12-13 (7-8)[v]	88(89)
Coconut-olive-safflower	(18)	37	10/9	+	+ 24[w]	89
Coconut-olive-safflower	(18)	37	10/9	–	- 10[w]	89

[a]Peanut oil (57 IV) has 40-45% *trans* and essentially 0 *cis*-9, *cis*-12-18:2.
[b]Serum cholesterol "fell" an unspecified amount when EFA was added to hydrogenated peanut oil diet. (cf. 75, p. 523).
[c]Hyperlipidemic male with atherosclerotic heart disease and healed myocardial infarction. Baseline total serum cholesterol ca. 300 mg %.
[d]Equivalent to baseline value.
[e]Female with cardiac neurosis, baseline total serum cholesterol 244 mg %.
[f]NC = no change. Considerably less than baseline value.
[g]Hypercholesterolemic male with atherosclerotic heart disease and healed myocardial infarction. Baseline total serum cholesterol ca. 343 mg %.
[h]Relative to control with 45 cal % fat and >500 mg cholesterol. Fats fed at 40 cal % with 28 mg cholesterol.
[i]Experimental fat (30 g) substituted for 68 g of carbohydrate in ca. 40 cal % fat "house" diet.
[j]Experimental fat (100 g) added to "low-fat base diet".
[k]Amount consumed, g/day.
[l]Experimental fat (98.5-100 g) added to "low-fat base diet." Hydrogenated fat reportedly had both *trans*-containing-nonconjugated dienes (7.5%) and conjugated dienes (4.8%).
[m]On 40 cal % fat diets, increase was in serum triglycerides.
[n]Fat (40 cal %) substituted for carbohydrate.
[o]Experimental fat (22.5 cal %) added to control diet containing 22.5 cal % butterfat.
[p]Experimental fat (45 cal %) added to fat-free control diet.
[q]Formula diet with 15 cal % protein, 41 cal % fat and 44 cal % dextrose. Cottonseed oil (114 IV) 56% EFA, 0% *trans* was control fat.
[r]Relative to control. Diets with cholesterol (742 mg/day) led to 24 mg % and 27 mg % increase in serum cholesterol of subjects on experimental and control diets, respectively.
[s]Relative to sunflower oil control and assuming ΔSC must be >12 mg % for significance. All fats at 33 cal %.
[t]PHSBO with 70% *trans* and 0 EFA with 10% SBO and 10% sunflower oil added (cf. 85).
[u]PHSBO interesterified 9:1 with safflower oil fed at ca. 40 cal % level. Control was interesterified olive, safflower and completely hydrogenated soybean oils (cf 79).
[v]Liquid formula diet with 40 cal % fat made up by blending coconut, olive/or hydrogenated olive and safflower oils plus ca. 220 mg cholesterol/day.
[w]Diets comparable to v. only one egg/day consumed to yield 250 mg cholesterol in diet. Increase and decrease in SC estimated from Figure 1A (89).

ment 1A was not flawless . . ." Careful scrutiny of the limited details of the experimental protocol (89) reveals that the liquid formula diets were "the main source of food" but they noted wide fluctuation of zero-point serum cholesterol levels (the "flaw" above was a zero-point range of ca. 24 mg % within the various groups; Fig. 1A, p. 11, ref. 89). Further, there was considerable serum cholesterol fluctuation noted throughout these studies (Fig. 1A and 1B, ref. 89). Of interest is the notation that the difference between the serum cholesterol levels with olive oil (A1) and the high *trans* plus cholesterol (A2-egg) was "systematical (confidence level >90%)" when the difference in values was ca. 24 mg % (estimated from Fig. 1A, ref. 89). Yet, earlier, Vergroeson (88) had attached significance to a difference of 12-13 mg % (estimated from Fig. 1, ref. 88) between the high *cis* and high *trans* diets and that difference was only ca. 7 mg % (Fig. 4) (89). Coincidentally, most of the key serum cholesterol differences in these experiments almost exactly match or are less than the corresponding zero-point ranges (15-24 mg%) in the two experiments. Another difficulty in interpreting these results is the lack of reproducibility in the key dietary groups. The high (34-35%) *trans* diets at ca. 10% EFA with added cholesterol differed widely in total serum cholesterol at 20 and 28 days (216 mg % in 1A vs 190 mg % in 1B, ref. 89). Similar comments are possible about the reproducibility in the high saturate diets with cholesterol at 10% EFA (193 mg % in 1A vs 209 mg % in 1B, ref. 89).

Gottenbos (personal communication) recently commented on these points, indicating that, in his opinion, the studies shown in Figure 1B (89) more nearly represent what occurs and that the experiment reported in Figure 1A (89) was "flawed". Thus, it appears that there was little, if any, "*trans*-effect" noted and that, within experimental error, *cis* and *trans*-monoenes appear to have similar effects with, at most, some trend toward but not equivalence to the effects of saturates (Fig. 4) when fed to humans under similar conditions. With the known problems in serum cholesterol analyses and the inherent variability in human serum cholesterol levels, attaching much significance (4,6,12,13,43,87) to such changes in the 7-12 mg % range appears questionable.

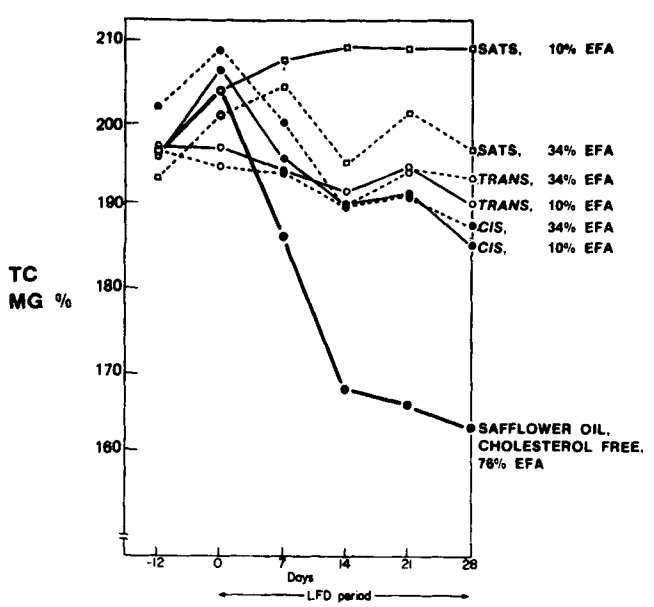

FIG. 4. Effect of cholesterol (220 mg/day) and EFA level on human serum cholesterol (mg %) in the presence of 37% saturates or 35% *trans*-monoenes (from hydrogenated olive oil) substituted for oleate. From Vergroesen and Gottenbos (89).

One can summarize the human studies to date in much the same fashion as the swine studies discussed earlier. In the carefully controlled and designed human experiments (79,83-85,88,89), it is not apparent that any significant "*trans*-effect" exists. As discussed, the other studies are equivocal and subject to interpretation with many variables other than *trans* implicated.

Other proposals implicating hydrogenated fats in CHD have been offered. These speculatively allude to human responses that might be anticipated from other studies. One of the leading and respected cardiologists, Sir John McMichael, has referred (90) to the *trans* fatty acids of cholesterol esters as having been shown (91) to be much more sclerogenic than those of fatty acids with *cis* double bonds. However, perusal of the tabulated data (91) reveals the following sclerogenic scores for the various fatty acid cholesterol esters (collagen score [max. 17 for 6 lesions]): stearate (11.8), palmitate (8.3), myristate (11.0), laurate (14.3), oleate (17.0), palmitoleate (17.0), linoleate (6.8), linolenate (6.8), elaidate (11.5), *trans, trans*-linoleate (17.0) and *trans, trans, trans*-linolenate (16.0). One can agree that McMichael's assessment (90) is correct for the *trans, trans*-(17.0) vs *cis, cis*-linoleate (6.8); however, the thesis breaks down for elaidate (11.5) vs oleate (17.0). The meaning for PHSBO is obvious as we have already shown that *trans, trans*-linoleate is a trace product of soybean oil hydrogenation. This is but one example of misinterpretation of published data that will undoubtedly lend additional confusion to the literature by further citation.

Another example of misunderstanding being promulgated by citation is the statement by Mann (87), "Another class of putative agents for impairing the hydroxylase consists of the '*trans*' fatty acids produced in oils by hydrogenation." This idea was attributed to a suggestion by Kummerow (15) that was based on the in vitro work of Egwim and Kummerow (92) where rats were fed PHSBO of <0.2% EFA and 48% *trans*-monoene as described by these authors earlier (93). Looking at this earlier paper (93), one is struck by the candor. "It is concluded from the results that the linoleate-deficient nature of the hydrogenated fat, rather than its high content of *trans* acids, would explain the high tendency of this fat to induce the accumulation of long-chain (n-9) fatty acids in the cholesterol esters and phospholipids of the tissue studied."

Yet, as noted earlier, in the swine studies reported by this group using similar PHSBO, this EFA deficiency was not considered a factor (44,48) nor did the authors (92) feel it affected their findings that led to the later citations (15,87) of questionable value. In retrospect, it is difficult to lend much credence to any of these interpretations disregarding the extremely low level of EFA in this type of PHSBO and attributing the various results to the ca. 50% *trans*-monoene present (reviewed in ref. 12) unless adequate EFA supplementation was provided (52).

A final example that deserves mention is the Enig et al. statement (94): ". . . the *trans* fatty acids in animal studies have been shown to have an effect on membrane permeability and to result in some altered enzyme functions." This was based on references to the use of the aforementioned minimal EFA-PHSBO by Hsu and Kummerow (95) and the Decker and Mertz studies (20) where SO_2 elaidinization reduced the EFA level to ca. 0.4%. In both instances, the "*trans*-effect," if it exists, certainly cannot be unequivocally separated from the well-known effects of EFA deficiency on such cell properties and functions. This point particularly has been stressed by Houtsmuller (5), who clearly demonstrated that glycerol trielaidate and trioleate had similar positive effects on rat heart mitochondria function in the presence, but not in the absence, of linoleic

acid. It was noted that the best values for the mitochondria function appeared in the trielaidate-supplemented group tempting Houtsmuller (5) to suggest that addition of elaidic acid offered the biomembranes additional possibilities for incorporation of a variety of fatty acids into phospholipids, leading to improved fluidity. For the interested reader, the additional discussions by Houtsmuller (5) regarding the biochemical transformations of *trans*-isomers are worthwhile.

Epidemiology is another approach that has been employed in attempts to understand the relationships of dietary factors and disease. Olson (96) has defined epidemiology and its limits most succinctly: "Epidemiology is the medical science that attempts to relate disease incidence and prevalence to variables in the host and environment. Although epidemiology can establish associations between an environmental or host variable and occurrence of a disease with a high degree of statistical probability, it cannot, of itself, prove a causal relationship between those variables and the disease. Such proof must come from other kinds of medical experiments." In addition to various dietary factors, he lists many other statistically valid associations with coronary artery disease including, among others, ". . . per capita usage of television" and with cancer including ". . . exposure to such industrial chemicals as. . ." (96).

Hydrogenated fats have been included in many epidemiological studies. Further, PHSBO and other hydrogenated fat use is increasing in almost every part of the world and it often has been tempting and easy to find a statistically valid association between consumption of hydrogenated fat and almost any increasing disease condition of the human race. The point deserves repeating, however, that what is found is an association and not a causal relationship. Some recent examples include a 1975 study of certain U.K. populations by Thomas (97) which goes through complicated analyses to show the association but not the causal relationship between mortality from CHD and consumption of hydrogenated fat in the period 1962-71. Further "appropriate" experimentation was urged to test for a causal relationship (97). On the other hand, Armstrong et al., also in 1975 (98), published a study on U.K. CHD mortality and commodity consumption which showed a significant negative correlation for CHD deaths and margarine consumption for the 1950-1967 period. These authors (98) urged great caution in interpreting the significance of any such associations. These two examples are illustrative of the differences that can be found from the use of epidemiological data. Kummerow's discussion in 1979 (99), though lacking data, claims a "correlation" has been developed from existing data on hydrogenated fat consumption and CHD death in many countries and further points to "differences" in the level of hydrogenated fat consumption and the *trans*-isomer level in blood cell lipids as support for the "correlation." However, statistical validity was not shown and the number of samples was undefined. Kummerow's discussion is part of a larger review dealing with ". . . angiotoxins as dietary risk factors in coronary heart disease" (99) where, in addition to the epidemiological "evidence" mentioned, it cites the earlier EFA-deficient swine studies (44,48) as supporting the idea that *trans*-isomers are "angiotoxins." The use of this term to describe hydrogenated fats based on such equivocal evidence suggests a lack of scientific objectivity and confuses and misleads those outside the scientific community. Particularly cogent is the testimony of Levy before a Senate subcommittee in 1979 (100) who noted that ". . . *trans*-fatty acids and other agents could contribute to the development of atherosclerosis, but much more research is necessary to access [sic] the magnitude of their contribution. In any event, it would seem unlikely, based on present knowledge, that angiotoxic agents are either the cause of atherosclerosis or the key to curing or preventing this disease in our society."

TABLE VII

Effect of Type and Level of Dietary Fat on Gross Liver Tumor Incidence in CD-1 Mice Fed for 17 Months[a]

	Liver tumors/group of 40 mice							
	Saline controls				Dimethylhydrazine			
Sex	Males		Females		Males		Females	
Fat in diet (%)	5	17	5	17	5	17	5	17
Type of fat								
Sat	7	2*	9	2	19	16	30*	17
Monoene	9	6	3	5	14	27*	18	12
Diene	5	9	5	0	10	23	13	24*
trans Mono	7	11*	4	2	18	11*	13*	13*
Triene	5	7	9	6	20	27*	29*	27
Total	33	35	30	15	81	104	103	93

[a]Brown (114).
*Significantly different ($P < 0.05$).

TABLE VIII

Effect of Type and Level of Fat on Gross Liver and Mammary Lesions in C_3H Female Mice Fed for 17 Months[a]

	Number of gross tumors or lesions/60 mice			
	Liver		Mammary	
Type/fat (%)	5	17	5	17
Sat	40	34	8	5
Monoene	32	46	2	9
Diene	49	40	4	3
trans Mono	38	37	1	7
Triene	32	20	2	5
Total	191	177	17	29

[a]Brown (114).

Turning to recent discussions of fat and cancer, Enig et al. (101-103) presented positive correlations between vegetable fats and especially *trans*-isomers and total cancer mortality and breast and colon cancer mortalities, and negative correlations with the same death causes and total animal fats from an analysis of selected data from various U.S. governmental publications. These studies were criticized by a number of authors (104-109) as erroneous and simplistic approaches to very complex problems. Enig et al. (110) corrected several glaring errors in the original publication (101), but insisted on the use of only certain selected data both as to fat consumption and cancer mortality to justify the associative relationship they perceive between hydrogenated fats and cancer. The end result is obvious. Proof of a causal relationship between cancer and hydrogenated fat has not been developed to date, even though these authors (101-103,110) speculated that results of very early work in this field by Tannenbaum (111) and Silverstone and Tannenbaum (112) could be accounted for by assuming that hydrogenated fat was a factor rather than only fat level, as suggested (111,112).

Recent work of Carroll and Hopkins (113), however, does not support the polyunsaturated vs saturated effect on chemically induced rat mammary tumors and has clearly shown that if a minimum EFA level is provided, then tumorigenesis is related to dietary fat level and not to fat type. Although these authors (113) did not specifically study PHSBO, their results were definitive enough to generalize their ideas regarding the influence of fat amount, rather than type, on tumorigenesis.

Subsequently, Brown (114) reported on a 17-month study of spontaneous and chemically induced (dimethylhydrazine) tumors in different strains of mice fed various fats at 5 and17% levels. The semipurified diets were supplemented with fats substituted isocalorically for sucrose. These fats contained saturates (59%), oleate (60%), linoleate (59%), *trans*-monoenes (35%) and linoleate (57%) plus linolenate (4%), respectively. No particular pattern of tumors emerged related to fat type and especially with respect to *trans*-monoene. Key results from Brown's work (114) are shown in Tables VII and VIII. Thus, under the conditions employed, there was no apparent *trans*-effect noted either in chemically induced or spontaneous tumors. Considering the complex multifactorial etiology of cancer, it seems highly unlikely that any simple relationship of this type will be found. Thus, attempts to develop the idea of a "carcinogenic" or "cocarcinogenic" designation for *trans*-isomers in hydrogenated fats seems no more objective than the use of the misleading term "angiotoxic" (99) considered earlier.

The evidence reviewed here does not show that PHSBO and the *trans*-isomers therein are implicated as lacking nutritional qualities, has not demonstrated that they contribute to CHD or cancer or that they are capable under normal EFA conditions of causing perturbations of cell enzymes or membranes. The idea that *trans*-isomers are "unnatural" and of recent occurrence in the food chain is false. Further, it is not evident from the results to date that the *trans*-isomers in PHSBO possess any unique, negative biological qualities in humans or animals when adequate levels of EFA are present in the diet.

These ideas are substantiated by a comprehensive literature review with 430 citations done for the U.S. Food and Drug Administration in 1973 by Tracor-Jitco (115) and again in 1976 by a Select Committee on GRAS Substances of the Federation of American Societies for Experimental Biology (116). The GRAS substances group reviewed the pertinent literature (90 references) and concluded: "There is no evidence in the available information on hydrogenated soybean oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used as a direct or indirect food ingredient at levels that are now current or that might reasonably be expected in the future."

Based on a concise review of some of the pertinent literature on hydrogenated fats in the period 1967-76, Juillet (117,118) has concluded that the determining factor with respect to cardiovascular problems is the total content of saturated or unsaturated fatty acids, and not their geometrical configuration.

At this writing, a summary of a report by an Ad Hoc Committee advising the Health Protection Branch of the Canadian government on the composition of special margarines has been issued (119). The detailed report is unavailable but apparently, this expert committee reached somewhat diverse conclusions regarding some aspects of *trans*-isomers in that they stressed the "uniqueness" of *trans*-isomers as to their metabolism in experimental animals and man, along with their similarity to saturated fatty acids. Without access to the total report, the precise source of this Committee's conclusions is unclear. They must have reviewed much of the literature discussed here, so one must conclude that their interpretations varied in part from those offered in this review. In most of the key areas, including swine atherosclerosis and human cancer, the Canadian group reached the conclusions presented earlier in this paper. They further noted: "The Committee encountered many areas of uncertainty with respect to knowledge of the metabolism of *trans* acids and of polyunsaturated fatty acids and their derivatives. We strongly recommend further research in this field, supported by government, industry and universities."

This, of course, is a laudable position and one that is difficult to question. However, from the information developed to date, the earlier statement of the Federation Select Committee (116) seems to be the more reasonable one and the recommendations of the Canadian group for limitations on *trans*-monoenes to ca. 15% of the fatty acid content of special margarines does not appear to be supported by data in the literature reviewed here. The same point can be made regarding other partially hydrogenated vegetable oil products. This recommendation from the Canadian Ad Hoc Committee apparently followed, in part, from the Vergroessen and Gottenbos (89) work already reviewed, where the results in humans are, at best, marginally significant if at all.

The committee noted, regarding *trans* fatty acids: ". . . some animal and human studies indicate they may elevate serum lipids." Perhaps a more adequate conclusion in agreement with the literature to date was their comment: "*Trans, trans*-18:2 excepted, and with adequate linoleate intake, there is no strong evidence that they are any more harmful than any other fatty acid class" (119).

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