The aetiology of the Spanish Toxic Syndrome: interpretation of the epidemiological evidence

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(Etiolog a del S ndrome T xico: interpretaci n de la evidencia epidemiol gica)

Summary

The disease (the «Spanish Toxic Syndrome») that caused some 20,000 people to be ill in Central and North West Spain in the summer of 1981 had not previously been known to medical science. Research into the cause of the disease has led many people to conclude that there was a toxic substance in some batches of oil that were sold for human consumption by street vendors. Laboratory studies have, however, failed to demonstrate toxicity in any of the samples that were recovered, no specific chemical that might have caused the disease has been identified, and the conclusion that the oil was responsible rests primarily on the epidemiological evidence. The purpose of this report was to review the epidemiological evidence to see whether the conclusion is justified or whether the possibility of some other cause needs to be considered. **Key words:** Spanish toxic syndrome.

Resumen

La enfermedad (el «Síndrome Tóxico») que tuvo unos 20,000 afectados en el centro y noroeste español durante el verano de 1981 no había sido previamente descrita por la ciencia médica. La investigación de la causa ha llevado a diferentes personas a concluir que existía una substancia tóxica en algunos lotes de aceite que se vendían de forma ambulante. Sin embargo, los estudios de laboratorio no han sido capaces de demostrar toxicidad en las muestras recogidas ni podido identificar agentes químicos que pudieran causar la enfermedad, y la conclusión de que el aceite era el agente responsable se basaba básicamente en la evidencia epidemiológica. El objetivo de este informe fue revisar la evidencia epidemiológica para averiguar si esa conclusión era justificada o si era necesario considerar la posibilidad de alguna otra causa. **Palabras clave:** Síndrome tóxico.

Introduction

The disease that caused some 20,000 people to be ill in Central and North West Spain in the summer of 1981 had not previously been known to medical science. Even in retrospect it has not been possible to identify any similar outbreak before 1981 and no similar cases have been detected since, either in Spain or elsewhere. Research into the cause of the disease has led many people to conclude that there was a toxic substance in some batches of oil that were sold for human consumption by street vendors. Laboratory studies have, however, failed to demonstrate toxicity in any of the samples that were recovered, no specific chemical that might have caused the disease has been identified, and the conclusion that the oil was responsible rests primarily on the epidemiological evidence. The purpose of this report is, therefore, to review the epidemiological evidence to see whether the conclusion is justified or whether the possibility of some other cause needs to be considered. In presenting the report I have assumed that the clinical, pat-

hological, and toxicological features of the disease are not open to question and have referred to them briefly, only in so far as they help to interpret the epidemiological data.

In preparing the report I have read in whole or in part the papers referred to in Appendix 1. Many of these are unpublished and were provided by the Spanish Ministry of Health, the World Health Organization's Regional Office for Europe, the Plan Nacional para el Síndrome Tóxico in Madrid, and by individual scientists in response to personal requests. I have also visited Madrid and had the opportunity of discussion with Dr. M.J. Clavera Ortiz and Dr. J. Martínez Ruiz who were known to be critical of the conclusion that the Toxic Syndrome was attributable to the consumption of toxic oil.

In summarizing the facts, as I believe them to have occurred, I have not given references if the facts can be found in the report of the World Health Organization's Working Group on the Toxic Oil Syndrome which met in Madrid in March 1983 (WHO Regional Office for Europe, 1984). For others I have given a reference. Interpretation of the meaning of the facts (for example,

the last paragraph of the section on 'Clinical and Pathological Features') is, however, purely personal.

Clinical and pathological features

Most of the affected subjects presented with an acute episode of fever, cough, and dyspnoea, which was often accompanied by myalgia, skin rashes, and changes in the chest x-ray suggesting non-cardiogenic pulmonary oedema. Eosinophilia was present in over 90% of patients by the third week and sometimes persisted for many months. Most patients recovered spontaneously within a few weeks. Myalgia, however, frequently persisted for many months and some 15-20% of cases progressed into a chronic phase that was characterized by symptoms and signs in many systems, including peripheral neuropathy, sclerodermatous changes, and severe salivary and lachrymal hyposecretion. A few patients presented only in the chronic phase, particularly in the later stages of the epidemic, and this complicates the analysis of the descending limb of the epidemic curve.

Some 2% of patients altogether and 3.5% of those admitted to hospital had died from the disease by 30.10.82 (Epidemiological Investigation Commission, 1984). At autopsy the lungs in the acute stage showed diffuse septal oedema and changes predominantly affecting the capillary endothelial cells with minimal evidence of inflammation; many other organs showed a non-necrotizing vasculitis. In the chronic stage, the most prominent feature was vasculitis of the small arteries with widespread fibrosis and atrophy of affected organs.

No evidence of any specific infection was found in life or at autopsy and immunological changes were few apart from an early transient increase in serum IgE.

These clinical and laboratory features exclude the possibility of a psychological origin and of a helminth infection and are reminiscent of some cases of periarteritis. The eosinophilia, which had suggested a helminth infection, weighs strongly against an infection of any other sort and suggests a toxic origin.

Epidemiological evidence

Characteristics of outbreak

1. The epidemic was first recognized early in May 1981, but cases are known to have occurred from early in April, if not sooner (Epidemiological Investigation Commission, 1984). From early May the increase in incidence was explosive and the peak of the epidemic occurred early in June.

2. The decline began about a week before there was any public suspicion that the disease might be due to the consumption of toxic oil (Epidemiological Investigation Commission, 1984) and the disassociation between the beginning of the decline and the beginning of public awareness that oil might be responsible for the disease is made more marked if an induction period of a few days to a week is required before the appearance of symptoms. Many of the new cases reported during the decline of the epidemic presented in transitional or chronic stages of the disease and it is not possible to tell from reported data how rapidly exposure to the causal agent diminished.

3. Geographically, the epidemic was almost confined to 14 provinces in Central and N.W. Spain. No cases occurred outside Spain, but a few (less than 200) occurred in other provinces.

4. Within the affected region, the epidemic spread progressively North West from Madrid to Leon.

5. Other features of the epidemic include:

a) the occurrence of clusters of cases close together in time within families;

b) the occasional recurrence of symptoms in patients who returned to their homes after discharge from hospital in the early stages of the epidemic;

c) the absence of secondary cases outside family clusters;

d) a slightly higher rate in women than in men;

e) a fairly uniform distribution by age except that no cases occurred in children under six months;

f) an absence of clusters associated with institutions that are characteristically affected in epidemics of infectious diseases (e.g. schools, military camps). A few outbreaks did, however, occur in convents (see p.14);

g) a concentration of cases in the industrial suburbs of Madrid, with a tendency to avoid both the most, and the least, wealthy areas.

Many of these features are consistent with either an infectious or a toxic aetiology; some, however, weigh against infection, particularly the absence of secondary cases [(5)c.], the rarity of cases in infancy despite a high incidence in women of reproductive age [(5)d.] and [(5)e.], and the social distribution [(5)f. and (5)g.]. Recurrence on returning home during the early stages of the epidemic also tends to favour a toxic origin from exposure at home.

The experience of Legionnaire's disease, which does not give rise to secondary cases and which was initially thought to be toxic in origin when an epidemic was first recognized in the USA, must, however, make one hesitate to exclude infection solely on these grounds.

Case-control studies

Evidence of Association

The idea that the consumption of a particular type

Table 1.

				Selection of subjects		Consumption of «street oil»		
Ref	Location	Date	Study unit	Cases	Controls	Cases	Controls	P-value
a	Madrid	? 1-8 June	Individ.	See Appendix	See Appendix	62/62 (100%)	4/62 (6%)	<.001
b	Nevas del Marqués	11 June	Family	27/30 affected families in town	108 families: 54 selected randomly 54 randomly after matching for size	27/27 (100%)	30/108 (28%)	<.001
c	Pozuelo de Alarcón	17-18 June	Family	Families of patients from Pozuelo district admitted to Clinica Puerto de Hierro	Neighbourhood families approached in defined order	42/48 (88%)	32/96 (33%)	<.001
d	Madrid	–	Individ.	–	–	7/7 (100%)	28/84 (33%)	<.001
e	Madrid	–	Individ.	–	–	9/9 (100%)	34/104 (33%)	<.001
f	Madrid	–	Individ.	–	–	8/8 (100%)	72/204 (35%)	<.001
g	Madrid	–	Family	–	–	52/58 (90%)	615/1725 (36%)	<.001
h	Chozas de abajo	15-22 June	Family	All affected families	Randomly selected families	19/19 (100%)	15/19 (79%)	>.05
i	Cerezo de arriba	19 June	Family	All affected families in village	Other families[1]	13/13 (100%)	25/44 (57%)	.002
j	San Cristóbal de Entrevinas	17-25 June	Family	? all affected families	Two sets: selected at random + 'matched'	10/10 (100%)	8/19[2] (42%)	.002
k	Bocigas de Perales	11-17 June	Family	All affected families	All other families	11/11 (100%)	22/33 (67%)	.03
l	Arconoda[3]	end July	Family	All affected individs.	All unaffected families	18/18 (100%)	9/21 (57%)	<.001
m	Colmenar viejo	26 June	Family	Patients from Colmenar admitted to Ramon y Cajal Centre	Neighbourhood families as (c)	16/20 (80%)	6/20 (30%)	.002

[1] Unclear how chosen: must have been most available as altogether 173 persons were included in 57 families out of 266 inhabitants.

[2] Only 9 replies to this question out of 10 matches controls.

[3] Note. Comparison is made between affected individuals and unaffected families. Different figures are given by Rigau-Perez in WHO, Regional Office for Europe (1984) report: i.e. 18 case families, all consumed street oil; 21 control families, 12 consumed street oil. The original report (1) also states that all the affected individuals except one (number not stated) at the locality of Lantadilla had consumed street oil as had the 12 people in the 5 unaffected families.

References (a) Casado-Flores et al (1982); (b) Rigau-Perez et al (1984), second of four studies reported; (c) Andres J, Segura A and Oñorbe J (1981) and Andres J, Segura A, Oñorbede Torre J and Spagnello E (1981); (h) Ministerio de Sanidad (1981,i); (i) Ministerio de Sanidad (1981,ii); (j) Ministerio de Sanidad (1981,iii); (k) Ministerio de Sanidad (1981,iv); (l) Ministerio de Sanidad (1981,v); (m) Servicio de Medicina Preventiva del Hospital Ramon y Cajal (1981).

of oil was the responsible factor arose from the results of enquiries about the background to the admission of paediatric cases to the Niño Jesús Hospital in Madrid. Around the middle of May, Dr.Tabuenca Oliver (1984) became convinced that the disease was toxic in origin and he sought the collaboration of the Institute of Hygiene and Safety at Work to look for heavy metals in biological specimens from affected children. By 1 June, he had come to believe that the disease was due to food poisoning, and in the first days of June, a questionnaire was administered to the parents of 62 affected children and 62 children with other conditions about the food that these children had consumed shortly before admission. The results showed a striking difference in the proportions who had consumed daily oil that was marketed and sold as olive oil In 5 litre plastic containers bearing no trademark or seal (100% against 6.4%) (Casado-Flores et al, 1982). This led to a public announcement on 10 June that oil of this type was responsible for the outbreak and to an offer, on 26 June, to exchange all

similar oil for pure olive oil at government expense. It is clear, however, that the oil to which cases of the disease have subsequently been linked has not always had precisely the characteristics describes by Tabuenca Oliver (some samples, for example, were sold in other containers with brand names) and the term «street oil» which will be used in the rest of this report, will imply only oil that was sold by street vendors, in street markets, or in small shops, that had bought the oil from street sources.

All the other case-control studies that were carried out were conducted after 10 June, when the concept that street oil was responsible for the disease was imprinted on the public mind, and this has to be borne in mind when assessing the results. Most, however, were conducted before the offer was made to exchange the oil (see table 1).

Unfortunately the first study has not been described in detail anywhere and some of its features are described differently in different accounts. It is, therefore, difficult

to assess its validity. A brief summary of the principal account, including some of the conflicting information, is given in Appendix 2. It is clear, however, that detailed questions were asked about a wide variety of foods, that the replies recorded were in general appropriate, that the results were internally consistent in that the control children who consumed very little street oil consumed more oil of other types, and that few other differences of possible importance were observed (Casado-Flores et al, 1982).

The results of 12 studies undertaken to check the conclusion of the first are summarized, together with those of the Niño Jesús Hospital study, in table 1. The results have been extracted from the original reports, whenever possible, but for four sets not included in the WHO, Regional Office for Europe (1984) report (references d, e, f, and g) I have had available only Kilbourne's (1985) report to the WHO Steering Committee. Different figures have been given in some instances elsewhere, usually because of the choice of different controls for comparison, but occasionally for reasons that are unclear. Table 1 includes data from all control series, combining, when available, the separate data for controls selected at random and those matched for selected characteristics.

All 12 studies confirm that street oil had been consumed in nearly every instance by the affected individuals or families. In each instance, the proportion that had consumed street oil was higher for the affected-families or individuals than for the controls. In 11 studies the difference was statistically significant and in 7 it was highly significant (P < 0.001). In sum, the proportion that had consumed street oil was 94% (232/248), while the proportion in the controls (weighted by the number of affected families or individuals in each study) was 40%, suggesting that the risk with a positive history relative to that with a negative history was increased approximately 25 times and unlikely to be increased less than about 17 times (95% lower confidence limit).

In one study (b) detailed enquiries were made about the source of the oil and the association was found to be much stronger when oil was considered that had been purchased from particular vendors. 0f 32 affected families who had bought street oil, 24 had purchased it from a vendor who was identified by his appearance, vehicle, and street cry, while only 5 out of 23 similar control families had done so. These figures give a relative risk for oil purchased from this vendor compared to that for oils purchased from other vendors of 10.8.

Similar associations with particular vendors were found in studies f (5 out of 8 who had purchased from street vendors against 17 out of 72) and h (9 out of 19 who had purchased from street vendors against 3 out of 15). The original description of the results of study h is, however, unclear and the results have been presented differently in different reports (see h and Scientific Steering Committee on the Toxic Oil Syndrome,

1984a).

Reality or Artefact

To interpret these results we have first to decide whether the association that has been observed with the consumption of street oil was real or whether it was an artefact of the method of enquiry. In principle, the results could have been produced artificially by bias in the selection of cases or controls, or, since the information was subjective, by bias in the way the interviews were conducted or recorded, or in the way the subjects responded to the enquiry.

Selection bias is effectively ruled out by the design of some of the studies which included all (or practically all) the cases that had occurred in a particular area or had been admitted to a particular hospital (studies b, c, i, j, k, 1) and by the selection of controls that consisted of all the unaffected families in an area (studies i, k, l), a random sample of them (studies b, j), or neighbouring families matched according to stringent criteria (studies b, c, j, m). As each set of studies led to results that were practically identical with most of those obtained by other means, we can conclude that selection bias cannot have been responsible for the results, unless a positive history of the consumption of street oil had been made a criterion for the diagnosis of the disease. This, however, cannot have been the case as many of the affected patients were diagnosed before 10 June, and even after 10 June the concept that street oil was the responsible factor continued to have the status of an unproved hypothesis for a considerable period.

Interviewer bias is more difficult to exclude as blind interviewing was not done and would not have been practicable. One way of testing for the possibility of bias in interviewing affected families or affected patients is to examine separately the results obtained for patients who were interviewed in the belief that they suffered from the syndrome, but were eventually shown to have had some other condition. It is not known, however, whether any such patients were interviewed. Subject bias leading control patients to under-estimate consumption can be tested for by comparing the results obtained from control histories with estimates of the frequency with which street oil was purchased that have been obtained in other ways. No objective figures for the consumption of street oil are, understandably, available, but the purchase of street oil by 30-40% of families in the affected strata of Spanish society seems not to have been unrealistically low (Scientific Steering Committee on the Toxic Oil Syndrome, personal communication). The one figure for the proportion of individuals who had consumed street oil that is out of line with the rest is the 6% for control children reported in the initial enquiry (Casado-Flores et al, 1982). This low figure is unlikely to be due to chance. It could have arisen if the hospi-

tal drew its patients from a relatively low consumption area; but it could reflect a lower intensity of questioning of the control patients.

The short intervals between the presumed period of exposure and the onset of symptoms (approximately one week) and before the interviews were conducted (seldom more than a few weeks) should have prevented the introduction of any major bias due to differential recall. Respondent bias could, however, have been introduced if the purchase of street oil was regarded by controls as being discreditable or was regarded by affected patients as being advantageous. The former may have been the case to some small extent, but seems unlikely to have affected the control histories materially. The latter is unlikely to have been influential early in June 1981 as the offer to exchange street oil for pure olive oil was not made until 26 June, while special economic aid was not provided until September.

I conclude that bias is unlikely to have influenced the results to any important extent. It follows that, as the differences in the consumption of street oil between affected families and cases on the one hand and control families and cases on the other are large, the association between exposure to street oil and the development of the disease must be largely real.

Confounding or Causality

It does not, of course, necessarily follow that the consumption of street oil, which was invariably said to precede rather than to follow the onset of the disease, necessarily caused the disease. It could have caused the disease or the purchase and consumption of oil could have been confounded with some other factor that was the direct cause, such as the consumption of other food or the use of another commercial product purchased in the same way. The distinction between these two explanations for an observed association is the central problem of many epidemiological studies. The distinction is seldom easy to make, but experience has gradually accumulated, which often enables it to be made with a fair degree of confidence. Case-control studies commonly provide much of the relevant evidence; but the distinction can never be made on the results of such studies alone.

The evidence from case-control studies includes the consistency of the association in different studies, the strength of the association, the quantitative relationship between the dose of the suspected agent and the estimated risk of developing the disease, the temporal relationship between exposure and the onset of the disease, and the existence of other associations and the inter-relationships between the different factors.

1. Failure to find a consistent association in different studies would weigh against a causal relationship. Consistency, however, accords with both a causal relationship and confounding and weighs in favour of cause only when it extends over different circumstances and different cultures. As all the present studies were undertaken in similar circumstances in one country, the fact of consistency contributes little apart from helping to exclude chance and bias, as discussed above.

2. The strength of the relationship is another matter, as cause becomes progressively more likely as the strength of the relationship increases. No specific limit can be set to the size of the relative risk that excludes confounding, but past experience suggests that confounding is seldom likely to be the explanation if the lower 95% confidence limit of the estimated relative risk is greater than 3. As, in the present case, the estimated relative risk is of the order of 25 to 1 with a 95% lower limit greater than 17 to 1, this certainly weighs in favour of the hypothesis that the oil caused the disease.

This estimate of the relative risk is, moreover, in all probability too low for two reasons. First, because it has not taken account of the greater risk associated with purchase from specific salesmen recorded in three studies and, second, because control families were sometimes chosen for comparison with affected families that were matched precisely for place of residence. It is understandable why controls were matched in this way, as the demonstration of a substantial difference between cases and controls matched for place of residence helps to reduce the possibility of confounding between the purchase of street oil and some special feature of the town or village such as the prevalence of infection. It may, however, have had the effect of grossly reducing the estimate of the relative risk if individual salesmen visited different villages and high incidence villages were chosen for study (as in studies i and l).

3. A progressive increase in risk with the amount consumed would also help to support a causal relationship. Quantitative estimates of food consumption are, however, difficult to make and the difficulty is enhanced with an 'item of food, like oil, that is not consumed by itself, but is used for many purposes in association with other foods. To obtain any worthwhile estimates detailed attention would have to be paid to the design of the questionnaire and pilot studies would need to be conducted to test the validity of the questions. It is not surprising, therefore, that few of the studies that were carried out during the heat of the epidemic obtained any quantitative data at all. In one study (c), an attempt was made to relate the proportion of the members affected in each of 48 families to the average amount of suspect oil consumed per month by each member of the family and to various indirect measures of oil consumption, such as the amount of salads, mayonnaise, etc. or the amount of fried food. All the correlations proved to be negative (i.e. the higher the proportion of members affected, the less the amount of oil consumed), but few details are given in the paper and it is not clear what was actually done. In four other studies, estimates were

made of the numbers of families or persons consuming different amounts of oil per unit of time. In three (studies h, i, and j), based respectively on 32 affected and 60 unaffected members of the same families, 13 affected and 25 unaffected families all of which were exposed, and 10 affected and 20 unaffected families, no material differences of any sort were observed between the affected and the unaffected. In the fourth (b), which has been reported in the greatest detail, a comparison was made between the oil consumption of 56 patients and 58 unaffected members of affected families, and the proportion of patients was found to increase progressively from 26% (9/35), when the consumption was less than a quarter of a litre a week, through 53% (27/51) when it was a quarter to a half litre per week, to 71% (20/28) when it was more than half a litre per week. One month later, however, (on 9 July) when further enquiries were made of a subgroup of families (32 affected and 23 control) who had used street oil, no association could be found between the risk of illness and the estimated weekly consumption. In sum, the lack of a consistent dose-response relationship weighs against a causal rôle for the oil, but in view of the difficulties of obtaining accurate information about personal consumption, particularly in the circumstances in which the enquiries had to be made, the weight to be attached to the finding can be only small.

4. Individual histories have indicated a latent period of a few days to two weeks between the first consumption of street oil and the onset of symptoms, but no evidence to denote a specific temporal relationship appears to have been sought in the case-control studies. Two (c and m) enquired about purchase «after Easter», two (b and j) enquired about purchase «after April 1st», while one (h), which did not apparently specify a date for the purchase of street oil in general, asked a subgroup about purchase from specific vendors in the last two weeks of April. The responses to these questions are compatible with a causal relationship, but do not strengthen belief in it.

5. In several of the studies, information was sought about other possible factors, including other items sold by travelling salesmen (b, c, m), the consumption of other foods (a, b, c, h, and j), exposure to household materials and domestic animals (b, h and j), and housing and general social conditions (b). Several associations were observed with potential sources of toxic material. In the initial study at the Niño Jesús Hospital (a), associations were found with spices, processed cheese, canned fruit, and canned vegetables (see Appendix 2). At Nivas del Marqués (b) a greater proportion of affected families than of control families were found to have purchased a particular shampoo also commonly sold by itinerant vendors (29.6% against 1.7% in the first study and 11.0% against 0.0% in the second) and a greater proportion of affected members than of healthy mem-

bers of the same families were found to have consumed salads (89% against 60%). Multivariate analysis, however, failed to show a significant association between illness in the family and the consumption of salads, even when street oil was omitted from the analysis. At Pozuelo de Alarcón (h) an association was found with the purchase of wine from street vendors (20/48 families against 22/96), but this difference, unlike that for the purchase of oil, ceased to be statistically significant when it was limited to families who had bought from street vendors since 1 April. In brief, no factor was found that was as closely or consistently associated with the syndrome as the purchase of street oil, or which differentiated as well between affected and unaffected families, and it is not possible to attribute the association with street oil to confounding with any of the many other factors that were examined.

Dr. Clavera Ortiz and Dr. Martínez Ruiz (personal communication) believe that the late Dr. Muro had observed a strong association with the consumption of tomatoes that had been grown in one part of the country, but data to support this hypothesis were not available for assessment.

Other epidemiological evidence

Hypotheses derived from case-control studies can be tested epidemiologically In two ways: 1) by seeing whether the results can be used to predict successfully the subsequent risk of developing the disease in people with different degrees of exposure to the suspect agent and 2) by seeing how well the postulated relationship fits the observed incidence of the disease in place and time. In the present case, the first method is not available, as new cases of the Toxic Syndrome ceased to occur in 1981, and we have to depend on the second method alone. This method can, however, provide crucial evidence, particularly when we are dealing with a disease like the Toxic Syndrome, which is not known to have occurred in any other place or at any other time, when it is unreasonable to suggest that the disease should have had more than one cause. In these circumstances, outlying observations that do not appear to fit in with the geographical and temporal distribution of the disease may serve to destroy the hypothesis or, if they can be explained, to provide strong evidence that it is, in fact, correct. Several outlying observations of this type that have been reported are, therefore, examined in detail (pp. 7 to 9).

Geographical Location

One of the most striking pieces of evidence is the localization of the epidemic to one part of Spain and the question arises how the oil sold In that part of the country

during the spring and early summer of 1981 differed from that sold elsewhere. The histories obtained from affected families and from street vendors operating in the areas indicate that most of the suspect oil was distributed by three suppliers (RAELCA, Aguardo El Prado, and JAP); but many other sources have also been suspected, either because the oils supplied were associated with the occurrence of individual cases of the disease or because they were found to contain unusually large amounts of anilide, indicating that some components had been imported for industrial use rather than for human consumption. Neither of the studies that were undertaken by the Special Investigation Team of the Interministerial Commission (1981) nor that by Clavera Ortiz (1984) succeeded in delineating a clear network of supplies that corresponded to the affected areas and this must weigh against the idea that a few batches of oil were responsible for the production of the disease. It cannot, however, exculpate street oil altogether. The many samples that were surrendered at the end of June in exchange for pure olive oil had many different characteristics and it is impossible, at this stage, to define precisely the characteristics of those that were and those that were not associated with disease. Moreover, the conduct of legal proceedings has clouded the issue, as it has been against the interests of both vendors and suppliers to allow themselves to be associated with the sale of any batches of oil that might be accused of being toxic. The intricacies of the trade in oil, some of which had been imported for industrial use and refined and sold improperly for human consumption, may eventually be sorted out by judicial enquiry; but at present it is possible to conclude only that, if the oil was responsible, it must have been because of the special toxicity of a relatively small number of batches that were imported, processed, and widely distributed in the first half of 1981, some of which were imported by RAPSA at San Sebastian, handled by RAELCA and refined by ITH In Sevilla or DANESA-BAU in Madrid. The identification of a few pathways by which identified batches reached all affected families would be strong evidence that street oil was responsible. This, however, has not been achieved. The failure to identify common pathways certainly weakens the case against street oil; but, in the peculiar circumstances of the trade, it does not prove that the oil was not responsible.

Temporal Distribution

Cheap brands of so-called olive oil have been sold by street vendors throughout Spain for many years and it follows that, if the epidemic was due to such oil, it must have been due to oil produced in a new way or supplied from a new source. This is compatible with the vast majority of the information obtained from affected families or individuals as practically all of those interrogated had bought and consumed new supplies of oil shortly before the symptoms of illness appeared. Specific examples include: 1) the 11 affected families in study k, all of which had purchased street oil during the last days of April, while the 22 unaffected families that had also purchased it, had purchased the oil before the beginning of the month, and 2) the outbreaks in three of the four convents that are describes in outline in table 2.

Some of this 'new' oil that was consumed by people who subsequently developed the disease can be traced back to five batches of denatured rapeseed oil that were imported as industrial oil between March and May 1981 and were subsequently refined and distributed during the epidemic in April, May, and June 1981, or shortly before it began (Special Investigation Team of the Interministerial Commission, 1981; Epidemiological Investigation Commission, 1984). The significance of this temporal association is, however, diminished by the inability to demonstrate that the distribution of the batches related specifically to the geographical area in which the disease occurred.

What was initially thought to be stronger evidence was the decline of the epidemic following the announcement that the disease was due to adulterated oil and the subsequent exchange of samples for pure olive oil at government expense. In fact, however, the decline in incidence had begun a week or more before the announcement was made (Epidemiological Investigation Commission, 1984), when the idea that street oil might be responsible for the disease was limited to a small group of research workers.

It must, therefore, be concluded that the temporal distribution of the epidemic adds little or nothing to the weight of evidence in favour of the hypothesis. It is, however, generally compatible with the hypothesis, with the possible exception of the one outlying observation that is describes below (p.19).

Outlying Cases

According to the WHO report (WHO Regional Office for Europe, 1984) less than 200 cases have been registered as occurring in people who lived outside the affected region, the great majority of whom have been found to have had meals in the affected region before the onset of their illness (Scientific Steering Committee on the Toxic Oil Syndrome, 1985 [personal communication]). Several such cases were referred to in the report of the WHO Regional Office for Europe (1984) and some of them have been described in detail by Posada et al (1985).

According to the latter authors the Toxic Syndrome is recorded in the Government's census of affected persons as having occurred in four families in Sevilla, which is approximately 300 km away from the affected region. Representatives of each family were interviewed per-

Table 2. Consumption of suspect ¨olive oil¨ and occurrence of symptoms in four convents

Convent	Population	Date	Purchase of oil	Use of oil	Ocurrence of illness
1		Mid-February	100 litres from a street vendor mixed with 20 litres previously purchased		
	23 nuns	February to beginning of May		Dressing for salads and vegetables	Unspecified number complained of general weakness, several of sligh fever and chest pain, treated symptomatically
	23 nuns	Early May to end June		Used for cooking as well because 'more nutritive'. All but 12 litres used.	
	23 nuns	End May			Typical cases of toxic symdrome. 20 affected, 8 of whom developed chronic symptoms with one death
	Chaplain	February to end May		1 meal a day	Unaffected
	Relative of Superior	February to end May		Ate very little oil, because on special diet for medical reasons	Unaffected
2	35 nuns	Early May	10 litres JAP oil from local shop		
	26 nuns and chaplain in retreat	15-25 May		5 litres consumed mainly for salads	13 nuns developed mild symptoms: cough, dyspnea, myalguam; 6 progress to chronic phase
	9 nuns	15-25 May		nil	Unaffected
	35 nuns and chaplain	25 May to end June		5 litres consumed mainly for salads	1 of additional 9 nuns developed acute symptoms progression to chronic phase
	56 laywomen	Early May to end June		Same meals as nuns but used soybean oil for dressings	Unaffected
3	43 nuns	May	20 litres JAP oil from same shop as used by Convent 2		
	42 nuns	Mid-May to end June		18 litres consumed as dressings for salads and vegetables	42 developed dyspnoea, myalgia. 9 progressed to chronic phase
	1 nun	Absent for 16 days of period			Unaffected
	70 laywomen	Mid-May to end June		Same meals as nuns, but used soybean oil for dressings	Unaffected
4		End April	Gift of 20 litres oil bought from street vendor		
	13 nuns	Early May to 31 May		Oil used for all purposes (salads, cooking, etc.). Taste poor, mixed with other oils. 2.5 litres to 1 litre soybean and 1 litre sunflower seed oil. 12.5 litres original oil consumed.*	3 developed acute symptoms diagnosed as Toxic Symdrome
	Physician) Gardener) Chaplain)	Early May to 31 May		Visited convent. No meals	Unaffected

*Use of oil ceased because physician suspected a possible connection between illness and the ingestion of oil.

sonally. Two families had visited the epidemic area at the time of the epidemic, when they had consumed oil of the suspect type. Two families had not.

One of the latter families consisted of a man, his wife and daughter, and a niece who was with them temporarily. Food was bought from local stores, not from tra-

velling salesmen, and all food products purchased had brand names that implied sanitary control. Meals were prepared by the wife and eaten at home. Food oil was acquired from the ITH oil refinery in which the man worked, his last allotment having been obtained sometime in May, 1981. This oil was used until the middle of June, when the family learnt through the news media that the epidemic might be due to street oil and that the refinery was thought to be implicated in its distribution. In June, both the wife and the daughter developed acute respiratory symptoms. The wife's illness was accompanied by a rash and dry mouth and was followed by temporary alopecia and weight loss, but no definitive diagnosis was made. The daughter's illness, which persisted for two weeks, was followed a month later by characteristic skin, joint, and muscular lesions, accompanied by marked eosinophilia (2,400 cells ml^{-1}) and was diagnosed as the Toxic Syndrome.

The other family had five members (a man and his wife, a son, a daughter, and a grandmother). Food products were again bought only from local stores and only with formal brand names. Meals were prepared by the grandmother and were eaten at home. The husband was employed as principal administrator of the same company that employed the head of the first family and he also brought oil home directly from his workplace. The last allotment was obtained in April or May, but the dates when it was first and last consumed are unknown. Only the wife became ill. In July she developed a rash, followed a week later by a dry cough and dyspnoea on exertion associated with a small pleural effusion. Three weeks later she developed skin, muscle, and joint symptoms, accompanied by an eosinophilia of 2,280 cells ml^{-1}, and later still she developed severe neuropathy and loss of weight. The illness, which was characteristic of the Toxic Syndrome, has been registered as such In the official census.

The ITH refinery, for which the two heads of families worked, refined denatured rapeseed oil for RAELCA, the oil distributor with whose products the epidemic has been most strongly linked. Supplies of oil were allocated only to the two men referred to above and no cases occurred in the families of any of the other 22 employees.

The evidence provided by these four Sevilla families is extremely persuasive. Its importance is not diminished by the fact that several members of the two affected families of ITH staff did not become ill, despite having consumed the same oil, as attack rates appreciably less than 100% were characteristic of the epidemic. This was observed, for example, in two of the convents listed In table 2 and can be attributed to individual differences in dosage and susceptibility. Its importance would be diminished if many sporadic cases had occurred outside the affected region which could not be related to the oil sold in the affected region; but

this appears not to have been so. The possibility, however, exists that the history of oil consumption was taken into account in registering sporadic cases, and the weight of the evidence that they provide would be greatly increased if it were possible to show that no case had been excluded, simply because it had not been possible to link it with the suspect oil. One way in which this might be done is suggested later (Appendix 3). Meanwhile, it would be helpful if a register could be published of all the sporadic cases with an indication of how each was connected with the suspected cause.

One observation that does not easily fit the oil hypothesis is the outbreak of disease In the convent at Casarrubios del Monte (referred to as convent no.1 in table 2). This differed materially from the outbreaks in the three other convents that were referred to earlier (p. 16). The facts, which are discussed below, are described differently, in some minor respects, in the reports by the WHO Regional Office for Europe (1984) and by Díaz de Rojas et al (1985) and, where they differ, I have preferred the account in the latter report, even though it dates from a later period, because the information was obtained in a special study that made use of convent records and individual clinical files, as well as the results of personal interviewing.

In convents 2, 3, and 4, the illness was confined to individuals who consumed the suspect oil (bought for two convents-from a local store at a specially low price and not directly from a street vendor) and the first symptoms appeared a few days after the oil began to be used. In convent 1, the situation differed in two ways: the oil was purchased in February, well before the outbreak of the epidemic, and only very mild and non-specific symptoms were reported for two months after the oil began to be used.

The occurrence of such mild symptoms during March and April can perhaps be attributed to the fact that the oil was initially used only for salads and vegetables, so that the amount consumed was quite small. On this basis, the development of typical signs and symptoms of the disease later in May is explicable by the fact that the amount consumed was increased early in May, when the oil began to be used also for cooking, on the grounds that it would be better for the nuns who had been unwell than the cheaper sunflower oil that had been used for cooking previously.

Whether the February purchase is consistent with the timing of the import and refining of the suspect oils is more difficult to decide. The village in which the convent was situated had a particularly close connection with the owners of the firm (RAELCA) which processed and distributed some of the suspect oil and, according to Díaz de Rojas et al (1985), there is no problem as the records show that «the first batch of refined and denatured rapeseed oil arrived In Spain in the first days of February 1981, and it was supplied to the convent by the importing factory by the middle of the same

month». There is a problem, however, if other reports are correct. In the initial report of the WHO Working Group (WHO Regional Office for Europe, 1984) the oil is said to have been purchased by the convent «in the first 10 days of February» and all records agree that the first shipment of suspect oil to RAELCA (preceding the five shipments referred to on p. 16) was received on 11 February (Special Investigation Team of the Interministerial Commission, 1981; Clavera Ortiz, 1984). The discrepancy would diminish if the Spanish word '*decena*', which was used in the original report and was translated in the WHO report as '*10 days*' is translated more correctly by the phrase '*10 days or so*'. But even so, it is difficult to attribute symptoms to oil purchased in this period. Most of the shipment of oil received by RAELCA on 11 February is said to have been returned to the importers as the colour and smell were bad and only 500 kg were retained. No record exists of what happened to this small batch, but it is not easy to see how it could have been refined so successfully and distributed so quickly that 100 litres of it could have been regarded as good oil and sold to the convent within a few days. And it is even more difficult to believe that toxic oil could have come from any other source at this time, when no other cases occurred in Spain until April. Repeated enquiries have failed to shake the belief of the nuns that the oil was purchased '*en la primera decena de Febrero*' and that no further oil was purchased until July (Martínez Ruiz, 1983), and the only suggestion that it might have been later is contained in a statement by Sr. López (1983) under legal examination that he had sold approximately 120 litres of oil received from RAELCA to the convent three or four times between the end of 1980 and the months of February or March 1981.

There remains the evidence of the few subjects who, while resident in the affected area, developed the disease, but could not be shown to have consumed any street oil it is unreasonable to suggest that a unique disease that has never been known to occur at any other time or any other place could have had more than one cause. If it could be shown that even one person who developed the disease could not have had any exposure to the suspected agent (either the oil or the toxic chemical that is supposed to have been in the oil) that would provide good grounds for exculpating the oil altogether. It is possible that some similar chemical may, in other circumstances, produce similar reactions elsewhere on another occasion (presuming, that is, that a chemical caused the disease) but we should not accept more than one cause for the cases that occurred in Spain in the summer of 1981.

According to the data in table 1, no evidence could be obtained that street oil had been consumed by 16 individuals or affected families out of a total of 310 (5.2%). This, however, is not the same as saying that they can-

not have consumed it. Some individuals may have consumed it without their knowledge when away from home, some may not have remembered accurately what they bought or ate, and some may have had reasons for suppressing the truth.

Kilbourne (1985), for example, reported to a meeting of the Scientific Steering Committee that representatives of two of the affected families in study (f) initially denied purchasing street oil, but that on re-interview a daughter in one family intervened to say that such oil had been purchased, while a neighbour reported that the second family had certainly bought the oil, but did not wish to admit it as they had resold the oil in their own shop. In both cases the positive histories were subsequently confirmed.

I have no information about the six affected families who are reported not to have purchased street oil in study (g) but further information has been given about the six similar families reported in study (c). Of the 10 affected individuals in the six families, six may have unwittingly consumed the suspect oil at home, as oil of unknown brand had been purchased in street markets, and three may have consumed the oil elsewhere. One man remains, in whom the diagnosis has been confirmed, who state's that he did not consume any oil at all. A register of affected subjects that are reported not to have consumed the oil, comparable to the register of sporadic cases outside North West Spain referred to previously, could provide a further useful resource for research.

Toxicological evidence

None of the samples of street oil that were collected in Spain in the summer of 1981 has been shown to contain chemicals that are toxic to animals in laboratory tests, other than aniline which was present in small amounts and produces, in larger amounts, effects in Man that are quite different from those observed in the epidemic. Nor has it been possible to produce samples that have toxic effects analogous to those observed in Man by reproducing the processes by which it is thought that oils imported as industrial oils were refined and blended to provide cheap substitutes for olive oil. This could be because street oil was not the cause of the epidemic.

Alternatively the experiments may have failed for technical reasons. First, species differences in susceptibility may make it extremely difficult to demonstrate toxicity in laboratory animals even when they are known to be toxic to Man. This has been true of several pharmaceutical products that have caused serious side effects in a substantial proportion of treated patients (for example, the appetite suppressant aminorex fumarate that was introduced in Switzerland in 1967 and the beta-blocker practolol that was introduced in the United

Kingdom in the mid-1970s) and, more pertinently perhaps, it was true of the substance added to margarine which is believed to have caused the outbreak of «margarine disease» in Holland in 1960 (Hermans, 1961).

Secondly, the long time that elapsed between the peak of the epidemic and intensive laboratory testing of the oil and the probable dilution of many noxious samples with other samples unrelated to the production of the disease may have so reduced the concentration of the toxic element that its effects could no longer be shown.

Thirdly, the agent may have been introduced into the toxic batches incidentally in, for example, the treatment of the oil before it was imported, so that the attempts to reproduce a toxic oil experimentally have necessarily failed.

Conclusion

Proof that a particular agent causes disease in Man can seldom be obtained conclusively, unless the disease is so mild that it is justifiable to attempt to reproduce it experimentally in humans. Occasionally it may be possible to test the idea by a properly designed experiment in prevention. More often, however, our conclusion has to be based on evidence that falls short of logical proof, and the correctness (or incorrectness) of the conclusion is demonstrated subsequently by our ability to control or to predict the future incidence of the disease. In these circumstances we are commonly guided by the results of experiments in laboratory animals. Positive evidence that the suspected agent produces an analogous disease in animals provides strong support for the idea that it is the cause of disease in Man; but negative evidence does not necessarily rule out the idea and should not be regarded as outweighing epidemiological evidence, if the epidemiological evidence is strong.

In the present case, toxicity tests in animals have not demonstrated the existence of any hitherto unknown toxic material in the suspect oil, and the question is, therefore, whether the epidemiological evidence is sufficiently strong to implicate the oil on its own. Review of this evidence provides no reason to suppose that the association that has been observed between the development of the disease and exposure to street oil bought between April and June is due either to bias or to confounding with any other factor with which the purchase and consumption of street oil was associated. This leaves causality as the most natural explanation. The lack of evidence for an alternative explanation is not, however, sufficient to justify concluding that the observed association reflects cause and effect.

Against the idea of causality is the fact that no clear relationship has been observed between the dose of oil consumed and the risk of developing the disease, the failure to obtain evidence of exposure for all affected subjects, the time relationships between the import of suspect oil and the purchase of refined oil and the development of typical symptoms by the nuns at the convent of Sta. Cruz de Casarrubios del Monte, and the failure to demonstrate geographical limits to the sale of specific batches of suspect oil corresponding to the region in which the epidemic occurred.

In favour of causality is the strength of the association deduced from interviews with affected and unaffected individuals and families, the generally close temporal relationship between the purchase and consumption of oil and the occurrence of disease, and the fact that so many (if not all) of the sporadic cases occurring outside the affected region were found to have been exposed to the suspect oil by peculiarities of their personal behaviour. In this respect, the four cases that occurred in Sevilla, about 300 km from the affected region, are particularly notable.

In my opinion the evidence against causality is inconclusive for the following reasons: 1) reliable estimates of the amount of an item of food that has been consumed, when it is not consumed by itself but is used in the preparation of other foods, are always difficult to obtain and estimates are likely to be particularly unreliable when questionnaires are designed hurriedly without an opportunity for pre-testing, as was necessarily the case in the emergency in which the Spanish case-control studies were undertaken; 2) not all personal histories can be expected to be accurate and some truly positive histories are nearly always recorded as negative; 3) the long latent period observed in one convent, during which only some minor malaise occurred, can be attributed to an increase in dose following prolonged exposure to an unusually low dose, while confusion in the reported dates can be postulated to explain the apparent incompatibility between the time the suspect oil was first imported and its purchase by the convent; and 4) the failure to define geographical limits to the distribution of toxic batches of oil corresponding to the distribution of the-disease can be attributed to the confusion caused by the anxiety of distributors and vendors not to be implicated as responsible for the epidemic.

It is, in contrast, difficult to see how the evidence in favour of causality could have been produced artificially; unless, perhaps, the evidence that so many of the sporadic cases had been exposed to the oil (such as the four cases that occurred in Sevilla) was due to bias against registering individuals as suffering from the Toxic Syndrome, if they were not known to have had any such exposure.

In the absence of laboratory evidence for a contaminant of oil that could have caused the characteristic pathology of the Toxic Syndrome, a conclusion has to

be based on the pathological and epidemiological evidence alone. The former strongly suggests a toxic origin for the disease. The latter strongly suggests that the disease was due to the consumption of oil that was sold as olive oil, but was actually made from other sources, in the course of which a toxic substance of unknown character was either introduced or formed.

There are, however, too many gaps in the evidence to allow the conclusion that oil was definitely the cause. Such a conclusion could, however, be reached, even In the absence of toxicological evidence, if some of the gaps were filled. In view of the very high relative risks estimated from the case-control studies it would, in my opinion, be a proper conclusion if it were possible to defi-

ne the sources of supply which explained both the temporal and geographical distribution of the disease'; or if it were possible to provide a satisfactory explanation of the events at the Casarrubios del Monte convent and to show that practically all the sporadic cases outside the affected region had had the opportunity of consuming the suspect oil and that there had been no bias in their diagnoses, produced by knowledge that the subjects had been exposed to the oil. Two steps that might help to achieve this aim would be the publication of a list of sporadic cases with a note of the extent to which they were known to be exposed and an investigation along the lines of that describes in Appendix 3.

Appendices

1. Sources of Information

Sources referred to in the text are starred. (NB: as in the original report)

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2. Niño Jesús Hospital case-control study

The most detailed account of the Niño Jesús Hospital study is that given by Casado-Flores et al (1982) in which it is stated that a dietary investigation was carried out among the parents of the children admitted to the hospital during the first days of June. The affected children were those among the first 46 affected families, totalling 62 in all, while the 62 controls were selected without any defined procedure from other in-patients or surgical out-patient clinics. At least 42 items of food and drink were enquired about and the proportions consuming them during the days prior to the children's first admission to hospital are shown in histogram

form. Very few numbers are given in the text and attention is drawn solely to the difference in the proportions consuming unbranded oil sold in 5 litre containers (100% against 6.4%), and other oils (cases: all other types 0%. Controls: branded olive oil, 22.5%; branded olive oil and sunflower oil 19.4%; sunflower oil, 19.4%; oil «home produced or straight from an oil press 16.1%; seed oil with a registered mark bought loose 14.5%)* and to the similarity in the proportion consuming running mains water, fresh fruit, biscuits and pastas, various types of pulse vegetables, eggs, green vegetables, fish, sweetmeats and cakes, yoghurt, chocolates and cocoa».

Examination of the histogram shows, however, several other differences between the groups, though all are smaller than the difference in the consumption of unbranded olive oil in 5 litre plastic containers. The largest differences were: various spices (cases 80%, controls 43%), processed cheese (54% and 0%), canned fruit (35% and 20%), canned vegetables (22% and 0%), pastas (22% and 42%), fresh cheese (20% and 42%), lamb (18% and 0%), and branded custard (17% and 56%).

Finally it is noted that the investigation continued to be carried out on successive children admitted for the same syndrome and that the findings did not vary when 124 with the syndrome were compared with 121 without.

In other accounts, some of the details differ. Tabuenca Oliver (1984), who was one of those responsible for the Niño Jesús Hospital study, lists many other questions and implies that patients were selected from (for example) those who relapsed quickly after discharge from hospital and households with a large proportion of sick members. According to him, 97% (not 100%) of patients had consumed the oil compared with 6.4% of the controls.

Rigau-Pérez (1984), in his summarized account of the study, states that food consumption histories were obtained from approximately 30 patients with the toxic syndrome between 1 and 4 June and from additional patients between 4 and 8 June. Altogether 124 cases and 124 controls were interviewed and all the affected patients had consumed the oil.

3. Investigation of cases of Toxic Syndrome in Sevilla

The evidence relating the occurrence of sporadic cases of Toxic Syndrome outside the main affected region carries great weight in helping to decide whether the consumption of oil from certain suspect sources caused the disease. It does so, however, only if it can be demonstrated that the diagnosis of such cases was not biased by knowledge of the history of exposure. One objective sign of the disease was the occurrence of substantial eosinophilia (2,000 or more eosinophils per ml). The possibility of the existence of such bias could be investigated by examining the records of the pathological departments of all Sevilla hospitals and listing all the patients who were found to have such blood counts between (say) April and August 1981. An independent medical review of the final diagnosis of the conditions from which the subjects were suffering and a comparison of the diagnoses after review with those in the register of cases of the Toxic Syndrome and of the histories given by any patients included in one list, but not in both, would indicate whether the possibility of diagnostic bias existed.

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*Oil consumption is specified only for 61 out of 62 controls; yet those who used seed oil with a registered trade mark bought loose are describes as «the remaining nine».

Addendum 8.6.87

New epidemiological evidence

In the 20 months that have passed since the-submission of my report on the Spanish Toxic Syndrome (Doll, 1985), further epidemiological evidence has become available: namely., that provided by four papers reported to the Liaison Group of the WHO Steering Committee which met In Madrid on 27 and 28 January, 1987.

Orcasur case-control study

The first paper gives the detailed results of a case-control study that was carried out In Orcasur, a working class neighbourhood in south Madrid, from 29 October to 6 November 1981 (Cañas and Kilbourne, 1987). The preliminary results had been reported previously in outline by Dr. Kilbourne and were included in table 1 of my 1985 report (reference f), when *street oil* was recorded as having been used in all 8 of the affected households in comparison with 72 out of 204 unaffected households. In this study 277 houses were visited systematically and information about house-hold oil consumption was obtained from 212. No one was available for questioning In 59 houses, residents in 4 houses bought no oil for household consumption, and residents in 2 «were apparently unwilling to provide data the interviewer considered reliable».

In the preliminary report, which had been available In the summer of 1985, 8 households were recorded as having reported cases of the toxic syndrome in at least one household member, but review of the reported illnesses subsequently showed that they met a strict definition of the syndrome in only 5. The sources from which oil had been obtained by these 5 households and by 207 households in which no definite disease was reported are shown in table 1. All 5 had used oil from travelling salesmen against 71 of the 207 (34.3%) unaffected households and all 5 had obtained oil from the *mercadillo* (a Saturday open air market) against 27 of the unaffected households (13%). The 3 households that had previously been classed as having affected members on the basis of inclusion in the official census of cases, but whose diseases have not met the strict diagnostic criteria now used, are classed as unaffected in table 1. In all 3, oil had been purchased from travelling salesmen, but it had

been purchased from the *mercadillo* in only one.

Late cases

The second paper is a preliminary report of cases that occurred several months after the epidemic had ended. The authors (Pasada de la Paz et al, 1987a), who had worked for the Plan Nacional para el Síndrome Tóxico and who subsequently reviewed data collected during the legal investigation of the epidemic, came to learn of a family in which cases occurred approximately 7 months after the epidemic began and a single case in a man whose symptoms began about 12 months after it began.

The present paper is limited to the description of the last case. The man complained of «diffuse myalgia, cramps, decreased motility of the elbows and weight loss» of increasing severity from June 1982 which led to hospital admission on 22 October when he was found to have an eosinophil count of 16,200 cells mm^{-3} and an IgE of 202 i.u. Typical signs of the chronic toxic syndrome were recorded with muscular atrophy, contractures of the upper extremities, and scleroderma and a skin biopsy showed increased collagen deposition and perivascular infiltration by mononuclear cells and eosinophils. He improved gradually on treatment with corticosteroids and was discharged on 23 January 1983. During the previous summer he had travelled constantly and, when in Madrid, had normally eaten in restaurants in the centre of the city. In April 1982 his mother was taken ill, and died on 5 October 1982 after having been diagnosed as having cirrhosis of the liver. From April to July 1982, the man lived in his mother's house using oil from a container that is said to have been typical of the sort associated with the outbreak except that the container was labelled. There is reason to think that his mother had used very little oil from this container having previously consumed pure olive oil that had been provided by her daughters. The oil, unfortunately, is not available for examination as it is being retained for use in a case for damages, which the man is trying to obtain from the oil merchant who supplied it.

Peripheral cases

The third paper provides a preliminary account of the results of detailed investigation of the cases recorded as occurring outside the 14 provinces that were principally affected (Posada de la Paz, 1987b), which, it had been suggested, might help to fill the gaps in the epidemiological evidence (Doll, 1985). All such cases have been included that are referred to in the official census of cases, in the list of cases maintained by the Ministry of Justice, and in the list of cases reported from hospitals outside the area that was compiled by the Ministry of Health in 1981.

Cases have been included only if they met the following criteria: for acute cases, the presence of a «typical radiographic pattern» (presumably in a chest x-ray) with an eosinophilia of 500 mm^{-3} or more or the finding of non-cardiogenic pulmonary oedema and vascular endothelial damage at autopsy; for chronic cases, one of the above accompanied by neuropathy, scleroderma, pulmonary hypertension, substantial weight loss or the sicca syndrome.

Altogether 268 cases that meet these criteria have been traced and 241 have been reviewed and classified in one of the following four categories.

1. Became ill outside the epidemic area and exposed only to oil obtained outside the area.　41

2. Became ill outside the epidemic area, but exposed to oil obtained from inside the area.　39

3. Became ill inside the epidemic area and exposed to oil obtained from inside the area, but moved outside the area before the diagnosis was made.　158

4. Incorrectly included in the list of cases because of a coding error.　3

A further 3 cases have proved impossible to classify and 24 cases are awaiting review. Twenty-three of the latter have province codes in the official census implying that the illness began in the epidemic area and are likely to be classed in category 3.

The clinical records of 29 of those assigned to category 1 have been obtained. These show that 10 satisfy the clinical definition of a case, but that 19 do not. Records of the remaining 12 are still being sought.

Of the 10 confirmed peripheral cases, 5 occurred in Vizcaya, which is located on the north border of the epidemic area, 3 occurred in Sevilla, 1 occurred in Badajoz, and 1 in Alicante. The three that occurred in Sevilla have been reported previously (see Doll, 1985 and Posada et al, 1987) and have all been associated with the consumption of suspect oil that was refined in the town. The remaining 2 are still being investigated but as yet no links with the suspect oil have been obtained.

Characteristics of oils related to the Toxic Syndrome

Table 1. Sources of oil in affected and unaffected houserolds (after Ca as and Kilbourne, 1987)

Sources of oil	Number of	
	Affected households (5)	Unaffected households (207)
Grocery store or supermarket	4	164
*Molinos	1	31
*Almacenes or a granel	0	15
Travelling salesmen		
i)　*Mercadillo	5	27
ii)　Door to door	1 } 5	45 } 71
iii) Outside Orcasur	0	3
Unclassifiable	1	18

*Molino, a small oil-processing facility associated with an olive farm; almacenes, an establishment selling wholesale; a granel obtained from bulk supply in own container; mercadillo, Saturday open air market.

The fourth paper, which has been submitted for publication (Kilbourne et al, 1987), provides a detailed account of a new attempt to see if it is possible to associate the occurrence of the disease with a particular type of oil in an objective and unbiased way. Samples of oil were obtained from two warehouses in which oils were stored that had been obtained from households in two contiguous towns in Madrid province (Alcorcón and Leganés) during the Spanish Government's oil exchange programme in June and July 1981. Contact was made with the families concerned and the clinical records were reviewed of all the cases that were reported to have occurred. Of the 195 specimens originally selected 14 were excluded because they were duplicates or because the family from which the oil came could not be located. Eighty eight of the remainder were rejected, because the oil did not come from a typical container or because there was doubt about its association (or lack of association) with a typical case of the disease, and the remaining 93 (29 from affected and

Table 2. Concentration of oleic acid anilide in oils from affected and unaffected families (after Kilbourne et al, 1987)

Household	Number with samples containing oleic acid anilide, μg per g					No. of households
	0	1-100	101-600	601-1200	1201 or more	
Affected	11	2	3	6	7	29
Unaffected	48	6	7	3	0	64
Total	59	8	10	9	7	93

64 from unaffected families) were coded and sent to the laboratories of the Centres for Disease Control in Atlanta, USA, where they were analysed in ignorance of the class from which they had come. The results are striking. Oils from affected families were characterized to some extent by differences in the content of at least four fatty acids and two sterols, but notably by the presence and the amount of aniline and three fatty acid anilides. For each of these four last chemicals the probability that the differences could be produced by chance was less than 1 in 10,000. The results for oleic acid anilide (which was slightly, but not significantly, more closely associated with the disease than the three others) are summarized in table 2, from which it is seen that the risk was increased approximately 19-fold ($13/3 \div 11/48$) when the sample container more than 600 μg of oleic acid anilide per g. of oil compared with that when the chemical was not present.

Discussion

The new evidence is of variable quality. That provided by the Orcasur case-control study strengthens the association with the consumption of «street oil», in so far as It provides a complete and publicly available account of one of the 14 case-control studies that had hitherto been describes only in outline and pinpoints a particular source for the oil (namely a Saturday *mercadillo)* rather than one associated with travelling salesmen in general; but it is not qualitatively different from that previously available.

The evidence from enquiring into the background of the few cases that appear to have occurred after the general epidemic was over could be of crucial importance, but that now presented is too incomplete to be of any material help.

There remains the evidence from enquiring into the background of people recorded as having developed the disease outside the affected provinces and that from a new examination of samples of the available oils. The first is still incomplete; but the results already obtained were enlightening. The fact that 95.6% of the cases that have been adequately investigated (219 out of 229) have been shown either to have occurred in people who had eaten in the epidemic area or not to met the criteria needed for a positive diagnosis and that it has been possible to demonstrate a link with the consumption of the

suspect oil in 8 of the remaining 10 cases (3 definitely and 5 because they occurred in a province bordering on the epidemic area) provides strong support for the idea that the disease was due to the consumption of certain specific oils.

The second piece of evidence strengthens it even more. Not only does it confirm the existence of a general association between adulterated edible oil and the development of the disease, but it provides this in an objective manner that cannot have been biased by knowledge of the presence of disease. It also greatly strengthens the epidemiological evidence by providing clear evidence of a dose response relationship, which had been lacking from the previous case-control studies, and, moreover, evidence of such a gross risk with high concentrations of anilide (nearly 20 times that observed In the absence of anilide) that the association is extremely unlikely to be due to confounding between the use of adulterated oil and some other hypothetical agent.

Conclusion

In my report, I concluded that the epidemiological evidence led most naturally to the conclusion that the consumption of oil that was sold as olive oil, but was actually made from other sources, was responsible for the disease, and that the evidence against causality was inconclusive. I added, however, that there were too many gaps in the evidence in favour of causality to allow the conclusion that oil was definitely the cause.

The new evidence has filled some of the gaps. First, it has provided evidence that the number of exceptional cases outside the affected area is extremely small and that, in a high proportion of the few that did occur, some special exposure to adulterated oil either did exist or can be presumed to have existed. Secondly it has provided objective and unbiased evidence of a dose-response relationship between the risk of developing the disease and the concentration of certain chemicals (anilides) in oil that are not found in any natural oil. Moreover the increase in risk with high concentrations is so great that it is most unlikely to be an artefact due to association with any factor extrinsic to the oil.

With the addition of this new evidence, I conclude that adulterated oil was the cause of the toxic syndrome.

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