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DATE: October 25, 1972

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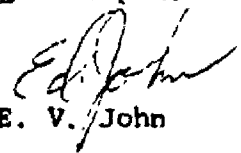
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The attached two articles on PCB appeared in Ambio, a publication of the Royal Swedish Academy of Sciences, dated August, 1972.

Authors are: Soren Jensen - "The PCB Story", and
Gensyu Umeda - "PCB Poisoning in Japan."


E. V. John

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Attachments

MONS 207807

AMBIO

A JOURNAL OF THE HUMAN ENVIRONMENT
RESEARCH AND MANAGEMENT

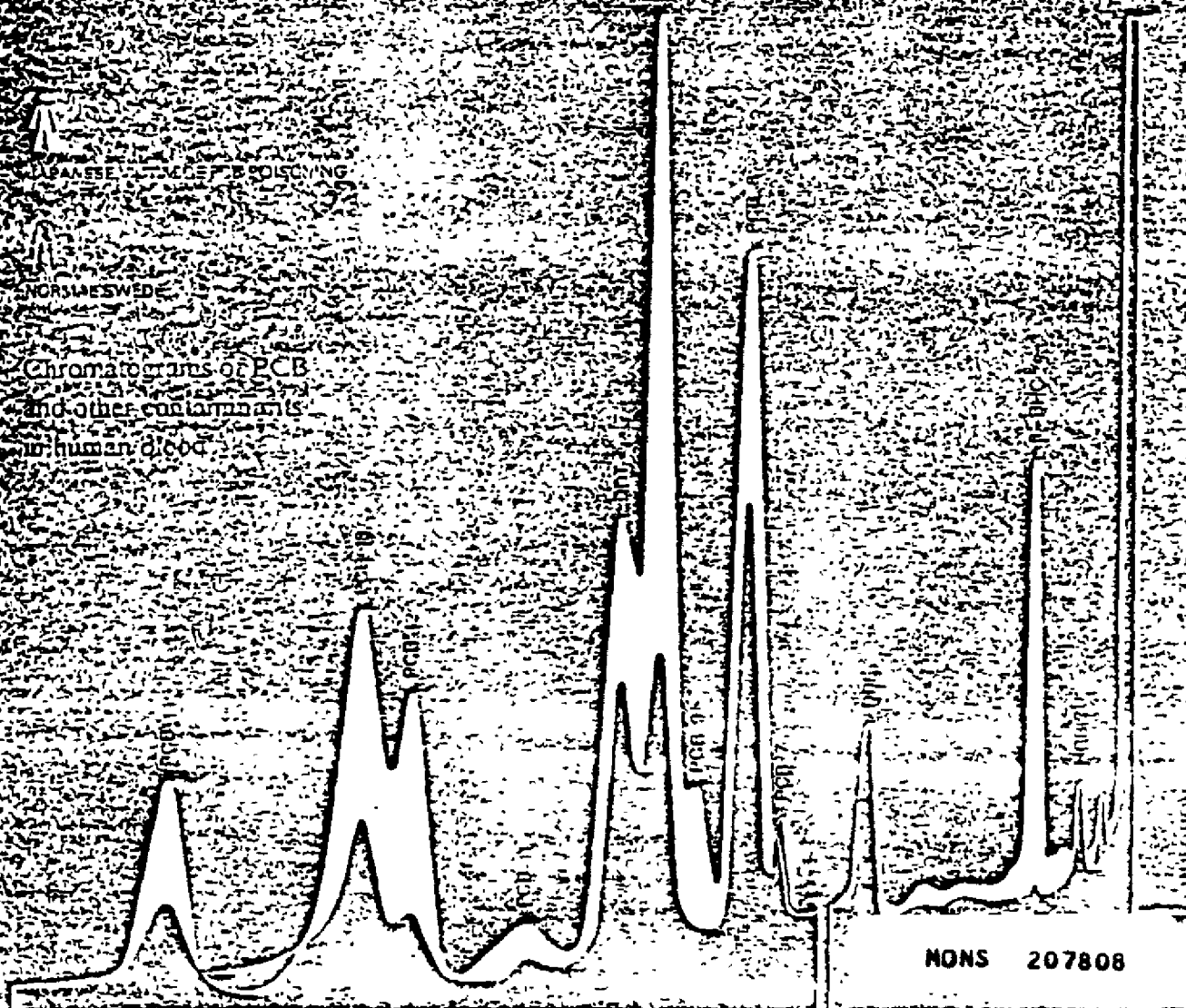
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JAPANESE AND SWEDISH

2

NORWAY

Chromatograms of PCB
and other contaminants
in human blood



MONS 207808

THE PCB STORY

BY SÖREN JENSEN

The accumulation of PCB in nature and thus in the food chains was unknown to scientists until 1966, although the substance had been used industrially since 1929. It was the Swedish chemist Sören Jensen who, when analyzing DDT accumulations in nature, accidentally found enormous quantities of unknown substances that were later on identified as PCB compounds. PCB constitutes perhaps the most stable group of organic substances in existence. It poses, therefore, a severe threat to life-forms.

In Sweden this discovery has led to a law prohibiting the use, import, manufacture and sale of PCB without permission from the authorities.

In this article Sören Jensen gives, for the first time, the complete story of his discovery of the substances. He also presents test results which show the PCB levels in blood samples from PCB-contaminated Japanese and other persons. The article can be read as a detective story with a moral. It can also be considered as an example of the kind of unpleasant discoveries we can expect unless environmental controls are applied to all dangerous substances before they are used extensively, before they can contaminate the Earth.

PCB pollution in nature was discovered at a rather late date, but considering the circumstances, it is perhaps surprising that it was discovered at all.

While the contamination of nature by PCB was not detected until 1966, the accumulation of DDT in nature was first recognized in the 1950's. During the late 1950's and early 1960's biologists and chemists became increasingly aware of DDT and mercury pollution.

The presence of these two substances in the food chains was discovered before the presence of other contaminants

such as PCB was revealed, largely because of the obvious connection in time and space between the use of DDT and mercury and the negative effects caused by them.

When farmers used mercury-treated seed, pheasants that lived in the fields and ate the seed died. When DDT was sprayed on plants to kill noxious insects, the birds living on the insects disappeared (1). In these cases, where there was rather clear evidence of cause-and-effect in situations that were relatively easy to observe, biologists could turn to analytical chemists for final proof of their hypotheses (2)

This was not and is not possible with a substance such as PCB that is intended for internal industrial use and enters nature through the "back door", i.e. with the emission of industrial smoke and sewage water.

An important aspect of this story is the development, in the late 1950's, of apparatus sensitive enough to detect a thousand millionth of a milligram of a substance. This amount is sufficient for analytical purposes if a standard is available, but is not enough for a structure determination of an unknown substance. Thus it was not until this time that the science of analytical chemistry possessed the tools necessary for the analysis and discovery of DDT.

DDT was first synthesized by the German O. Zeidler in 1874 and was first introduced as an insecticide shortly after World War II. But even if DDT had been put into use immediately after its invention, its contamination of nature probably would not have been recognized any earlier. And as a consequence, there would have been an 80-year accumulation of DDT to deal with instead of a 15-year-old one.

In 1964, the department of analytical chemistry at the University of Stockholm, where I was employed at the time, was required by the Royal Swedish Commission on Natural Resources of 1964 to estimate the levels of chlorinated pesticides, especially DDT and its metabolites, that were present in human fat tissues. Shortly thereafter the project was expanded to include wild life samples.

MYSTERIOUS SUBSTANCE DISCOVERED

The work started in October 1964. The analytical method used at the beginning was taken from available literature, but was later on modified slightly (see Experimental).

As DDT is fat soluble, dried homogenates of muscle samples were considered to be suitable for the experiments. Ether extracts from the samples were cleaned up by means of thin layer chromatography on microscope slides. The final fat-free extracts were injected on a gas chromatograph fitted with an electron capture detector. However, it is well known that with this system positive identification is not possible because several different compounds may have the same retention time on a single column. The risk of making a wrong identification is minimized by the use of double column systems, but it is never fully avoided. Although the detector is very sensitive to chlorinated hydrocarbons, it is by no means sensitive exclusively to such compounds. Thus many substances containing oxygen or phosphorous have shown quite good responses.

When the analyses were carried out along the lines set forth above, the work often resulted in chromatograms as

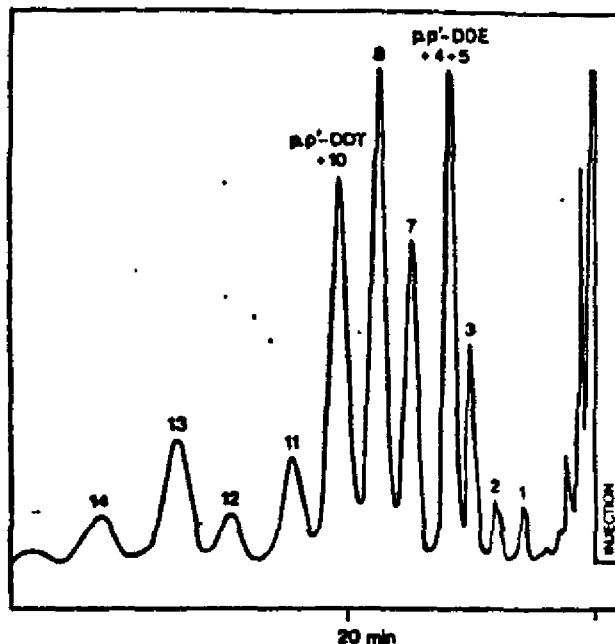


Figure 1. Gas chromatogram from purified extract from white-tailed eagle. All peaks but DDT and DDE were unknown. Column: SF 88 4 % on Gas Chrom P (HMDS-treated). Oven temperature ca. 190°C. Nitrogen flow 30 ml/minute.

shown in Fig. 1. Only p,p'-DDE and p,p'-DDT could be identified by means of their retention times. Among these two there were numerous peaks whose retention times did not agree with any of the known chlorinated pesticides. They were totally unknown to us. When a different gas chromatographic column was used the peak that had been assumed to be DDT was sometimes shown to be an unknown one.

COLLABORATION WITH ECOLOGISTS

Often up to 14 peaks could be found in a chromatogram of extracts from a pike and as they were not described in the literature the possibility of a pure analytical solution of the problem was at that point impossible. To get a final solution it was necessary to collaborate with ecologists. The first hypothesis to consider was that the unknown substances might belong to the common natural products. Therefore the Swedish Museum of Natural History in Stockholm collected pikes for analyses from Skåne in the south to unpolluted Lappland in the north. But the farther north

the origin of the samples, the lower were the levels of the unknown substances; thus it was clear that those substances did not occur naturally in pike.

The mercury problem in Sweden had just been recognized, and some organic mercury compounds were shown to possess a high response on the electron capture detector, so the museum collected pheasants, one of the species suffering most from the mercurials. However, it was shown that these birds did not contain the unknown substances. Could they possibly be unknown metabolites of the chlorinated pesticides? To check this hypothesis, one feather of each white-tailed eagle specimen was obtained from the museum's collection from 1888 up to present and the feathers were analyzed. The unknown substances were present already in 1942, but chlorinated pesticides were not used until 1945, consequently the unknown substances could not be metabolites of these pesticides.

Fig. 2 shows a series representing different levels of a food chain, including young salmon, pike and white-tailed eagle. Both DDE and the unknown substances increased up through the chain. At the same time there was a shift of the relative intensity of the unknown substances towards the late numbers in the chromatogram. But this was not especially pronounced, indicating that all these compounds must be very stable. It should also be noted that the aquatic ecological system was quite different from the terrestrial system represented by the elk.

There was a possibility that these unknown compounds could be metabolites of other pesticides such as the phosphorous insecticides and the chlorine-containing herbicides. However, neither treatment with alcoholic potassium hydroxide, nor concentrated sulphuric acid changed the chromatogram, except that the DDT and DDD were dehydrohalogenated by the alkali treatment and the epoxy pesticides disappeared during the sulphuric acid treatment. These treatments indicated that the unknown substances did not contain anything but hydrogen, carbon and halogens.

PCB IDENTIFIED

Fortunately for this investigation a white-tailed eagle was found dead in the archipelago of Stockholm and could be examined. It contained enormous amounts of the unknown substances. Thus the extract from 1 g of the muscle had to be diluted up to 5 l if a 10 μ l injection on the gas chromatograph should be within the linearity range of the electron capture detector. 20 mg of the fat was run on the microslide thin-layer plate as mentioned in the experimental part, and the eluate from the plate was evap-

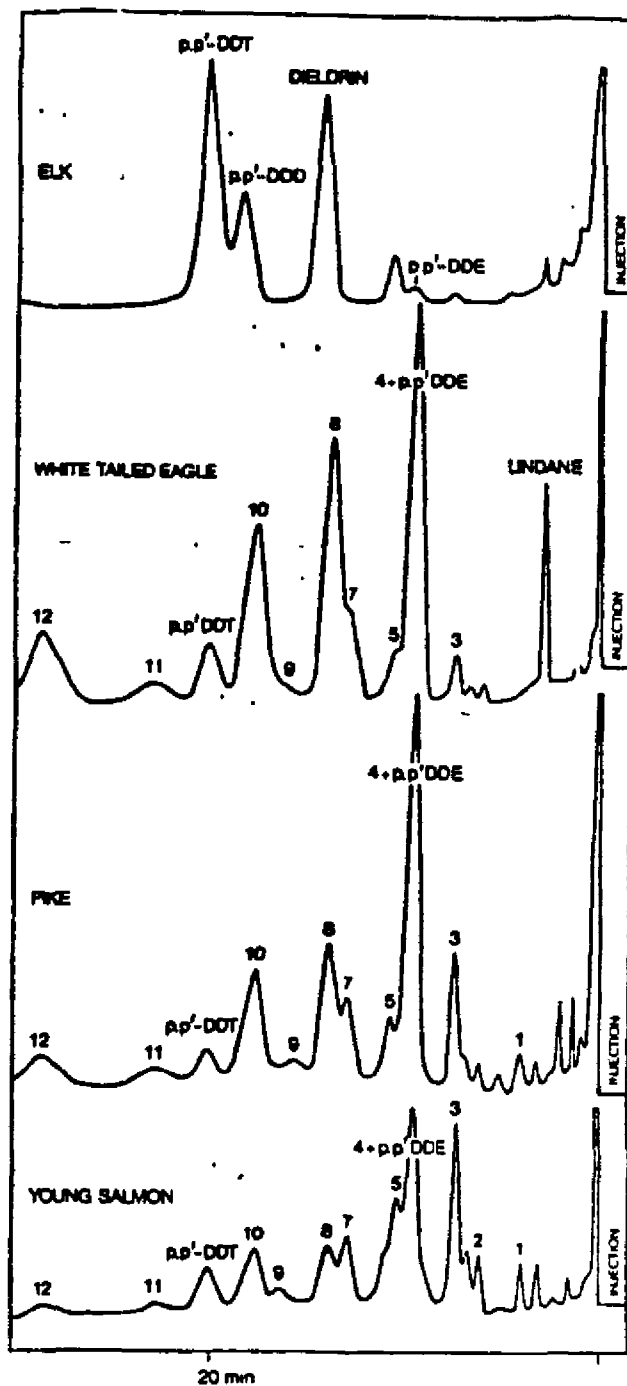


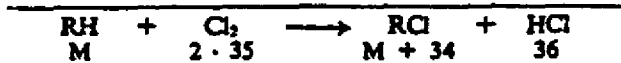
Figure 2. Gas chromatograms: residue analysis of body fat from different ecological levels. Column: QF 1 + SF 98 mixture.

orated to 50 μ l. This solution was then injected into the combined gas chromatograph-mass spectrometer. Figs 3a and 3b the chromatogram and one of the mass spectra. The mass numbers equal to the molecular weights of the unknown peaks could be read to 324, 358, 392 and 426. Astonishingly enough, the molecular differences were constantly 34 mass units. This constant difference for the first time showed that there was a relationship in the origin of the unknown substances. The presence of chlorine could easily be recognized from the unusual isotopic pattern—75 per cent is ^{35}Cl and 25 per cent is ^{37}Cl , which gives two molecular peaks two mass units apart with the relative intensity of 75:25. With two chlorines per molecule the intensities will be 57 per cent (two ^{35}Cl), 37 per cent (one ^{35}Cl and one ^{37}Cl) and 6 per cent (two ^{37}Cl) respectively. For x chlorine per molecule there will be peaks for $M + 2x$ ($x = 0.1.2.3.....$ where M is the molecular weight calculated by chlorine as ^{35}Cl).

By means of this isotopic pattern the following relationships for the unknown peaks were postulated:

Molecular weight:	324	358	392	426
Chlorine per molecule:	5	6	7	8

An explanation for the relationship between the unknown compounds can be read from the following equation (RH is a hydrocarbon):



The molecular weight of the parent hydrocarbon (PHC) could thus be calculated:

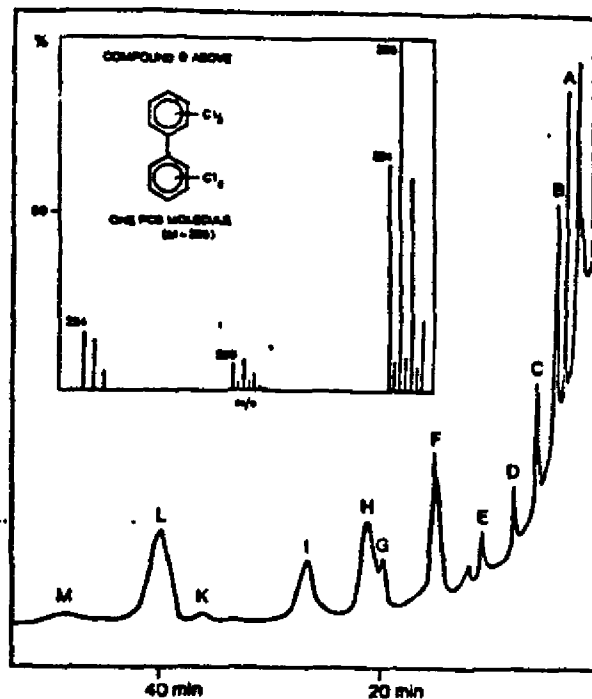
$M_{(\text{PHC})} = M - x \cdot M_{\text{Cl}} + x \cdot M_{\text{H}}$, where M is the molecular weight of the compound having x chlorines per molecule and for $M = 426$ and $x = 8$ chlorines:

$$M_{(\text{PHC})} = 426 - 280 + 8 = 154$$

(of course all the unknown substances will give the same value for PHC).

The most plausible formula for a hydrocarbon with a molecular weight of 154 is $\text{C}_{12}\text{H}_{10}$, which is only satisfied if the parent hydrocarbon is biphenyl, and logically the unknown substances were polychlorinated biphenyls, which have been given the name PCB.

The identity was later fully verified by injection of Clophen A 50 (a synthetic chlorinated biphenyl mixture) on the mass spectrometer. Furthermore, extensive gas chromato-



Figures 3a and 3b. Gas chromatogram fr combined mass spectrometry-gaschroma graphy of white-tailed eagle extract. Molecular weights for A, B, C, D and E: 282, 310, 324 and 338 (saturated hydrocarbon); 318 (DDE). G, H, I, L and M: 324, 358, 392 and 426 (chlorinated biphenyls with 6, 7, 7 and 8 chlorine atoms).

graphic investigations proved that synthetic PCB gave peaks with retention times identical to those of the unknown peaks from the white-tailed eagle and the other species investigated.

It took about two years to answer the question of the mysterious peaks of the unknown substances in the chromatogram. Thus PCB was finally identified in 1966 (3).

In the past we have learned that extremely persistent substances are potential contaminants of the environment and that the risk that they might accumulate via food chains increases if the substances are also fat soluble, such as DDE and PCB, or if they can react with the proteins in the organisms, such as heavy metals and organic mercurials. A valuable medium in an objective investigation of environmental pollutants might be digested sludge from sewage treatment plants, which contains both lipophilic substances and pr

teins and can thus enrich certain types of contaminants in domestic and industrial wastes. As digested sludge has been exposed to microbiological attack, only the most stable substances can survive to be environmental pollutants. Compounds may even be converted to stable metabolites with higher toxicity than the primary contaminants, as is the case with the microbiological methylation of mercury.

This system should prove valuable in the objective search for unknown pollutants. If sludge had been analyzed earlier, PCB might have been discovered as the result of a systematic search instead of by accident.

After the identification of PCB was finally made, it became apparent that many DDT analyses made in the past might be wrong because of PCB interference and it seemed probable that the risk for DDT contamination of nature had been overestimated. Instead, analyses had indicated contamination by PCB. This is true for analyses done by means of the methyl silicone and Apiezone columns often used up to 1965. In these old columns *p,p'*-DDT, especially, has been shown to overlap with PCB 10 (the last major peak). Thus the DDT apparently present in the chromatogram from the white-tailed eagle shown in Fig. 1 is actually PCB 10. This can be seen by means of the QF 1 column that is now often used (4).

WORLD-WIDE PCB RESEARCH

Since the discovery of PCB accumulation in nature was published in 1966, the presence of PCB has been reported in organisms from all over the world, and the subject has been reviewed by many authors: Risebrough *et al.* in 1968 (5), Risebrough in 1969 (6), Veith *et al.* in 1970 (7), Gustafson in 1970 (8), Peakall *et al.* in 1970 (9), and Reynolds in 1971 (10). Many attempts have been made to analyze PCB in the presence of the chlorinated pesticides, but no really satisfactory method has yet been described. The same is true with the problem of the quantification of PCB. As PCB is produced by direct chlorination of the aromatic hydrocarbon biphenyl, numerous homologues and isomers are found. 210 different substances are theoretically possible, and if the chlorine is distributed as evenly as possible on the two phenyl rings, 104 are still possible. Recently Sisson and Welti (11) have, in an excellent work, identified 69 different compounds in a PCB product used in industry by means of mass spectrometry (MS) and nuclear magnetic resonance spectrometry (NMR). In a normal column used in residue analysis, no more than 20 peaks are seen, and it is obvious that several peaks come from the overlapping of different compounds. Nevertheless, the mistakes made are

small in relation to the significance of the biological experiments.

CONTAMINANTS

Vos *et al.* (12, 13), found that not all commercial polychlorinated biphenyl preparations had the same toxicity. Significantly higher mortality, liver necrosis and chick oedema-like lesions were found in two out of three preparations tested. They found an active factor in a polar fraction and they concluded that the structures were tetra and penta chlorodibenzofurans. These compounds might have been formed from a phenolic contaminant in the PCB in the same way as 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (to which they are related) is formed from 2,4,5-trichlorophenol.

STABILITY

PCB is an extremely stable mixture against both chemical and biological attack. Thus it can be boiled with potassium hydroxide in ethanol or fuming sulphuric acid without degradation. It is reported that PCB is dechlorinated by means of ultraviolet light (14). PCB is said to be highly non-flammable if the chlorine content is above 4, but many authors have reported that it is easily degraded at elevated temperatures. Thus Karlsson and Rosén (15) found that around 950°C PCB will be destroyed within a few seconds. Ordinary oil burners, however, obviously are not equipped to do this, or else it would be difficult to explain the presence of PCB in the air, especially around big cities.

Recently it has been shown that PCB wastes in oil can be destroyed in existing industrial facilities through the burning of oil at 1,400°C with a deficiency of oxygen. This process is used in a cyclic system for the manufacture of ammonia as well as for other purposes. With this method factories can use waste oil containing PCB in their production processes, and can, moreover, function as destruction sites for PCB by mixing additional PCB with this oil.

The waxy layer on the needles of pines around Stockholm often contains PCB. This can be explained by assuming that when waste oils (which often contain around 50 ppm of PCB) are burnt, the PCB (at least to some extent) passes through the chimney, and into the environment. Another source of PCB might be the burning of solid wastes. The discharge of used PCB into the sewage treatment plants is indicated by the presence of PCB in digested sludge from these plants. That the biological stability of PCB is high in relation to DDT is seen in digestion experiments. When DDT and PCB are added to activated sludge

and digested anaerobically, DDT will be metabolized—mainly to DDD but also to more polar substances within a few days, while PCB remains intact after a 40-day period.

It is this stability of PCB that makes it useful in industry. In 1929 the electrical industry introduced PCB in capacitors and high voltage transformers because of its good insulating characteristics and because PCB is non-inflammable. PCB is now used in a number of different paints to make them resistant to chemicals and oxidation.

USE OF PCB IN SWEDEN

PCB is not produced in Sweden, but the annual import of bulk material amounted in 1969 to 600–700 tons, about 12 times the import of DDT. This situation was probably representative for many industrialized countries. Most of it was used as non-inflammable oils in capacitors and transformers. PVC and chlorinated rubber paint contained 5–8 per cent of PCB to increase the stability against chemicals and oxidation. Beside this, there is a 3–5 per cent addition to paints used on boats below the waterline. 80–85 per cent of all small boats and 50–60 per cent of the larger ships in Sweden have used such paints.

Gradually, it has been discovered that PCB occurs in a number of products in which there was no reason, earlier, to suspect its presence. Recently we found that large quantities of PCB were present in certain carbonless reproducing paper sold on the Swedish market (am NCR). This paper was used in forms which are prevalent in banks, libraries, etc. The PCB content was about 12 g per kg paper, which means that an original plus two copies in A-6-format (10.5 cm × 14.5 cm) contained about 110 mg PCB. PCB is no longer present in such paper.

The stability of the substance that makes it so attrac-

tive for industrial use is the same property that causes the accumulation of the substance in ecosystems and the food chains.

BIOLOGICAL EFFECTS

The acute toxicity of PCB is not very high. There is a relationship between toxicity and chlorine content which has led to different maximum allowable concentrations with a higher number of chlorine atoms, the maximum allowable in air is 1 mg/m³ air, and for the kinds of PCB that have a lower chlorine content it is 0.5 mg/m³. No maximum allowable level for PCB contamination in food has been established yet.

A typical symptom of PCB poisoning is fatty degeneration of the liver. One case of mass death of salmon fry at a Swedish hatchery showed this typical picture and the PCB source could be traced to anti-fouling paint used in the hatchery.

Effects on workers handling PCB have been reported and include various damage to the skin, apart from the liver degeneration mentioned above. It is estimated that the average Swede has a concentration of PCB of around 3–5 mg per kg body weight.

Damage to chicken embryos and inhibition of hatching occurs when 0.01 ml of PCB is injected into eggs. These experiments have not been followed up to test the lowest level at which these effects occur.

PCB (and DDT) are now known to activate the metabolic enzymes of the liver. The consequence of this is an increased breakdown of certain reproductive hormones which could result in changes of the reproductive rate in animals and man.

PCB accidents have been reported from the USA and Japan. In Japan (16), leakage from a corroded pipe containing PCB that was being used as a heat-transfer oil resulted in the contamination of the rice oil with up to 2,000 mg of PCB per kg. Thousands of people who ate the contaminated rice suffered from chloracne-like skin eruptions.

Just before people fell sick, one million chickens suffered from chick edema-like symptoms and about 700,000 died. The chicken food originated from the same factory.

Several thousands of persons are said to have suffered from PCB poisoning in Japan, although only 1,000 patients were officially reported to have been suffering from the disease, which is called "Yusho". Symptoms are skin eruptions, pigmentation, visual disturbances, abdominal pain and fatigue. Only a few patients recovered, since no treatment

Table 1. Annual PCB use in Sweden

Material	Unit size	Total amount of PCB
Capacitors	20 and 2–4 kg	450–500 tons
Transformers	500–2,000 kg	
PVC and other paints	1,000 tons	55 tons
Boat paint	400 tons	15 tons
Insulation paste	160 tons	35 tons
		555–605 tons

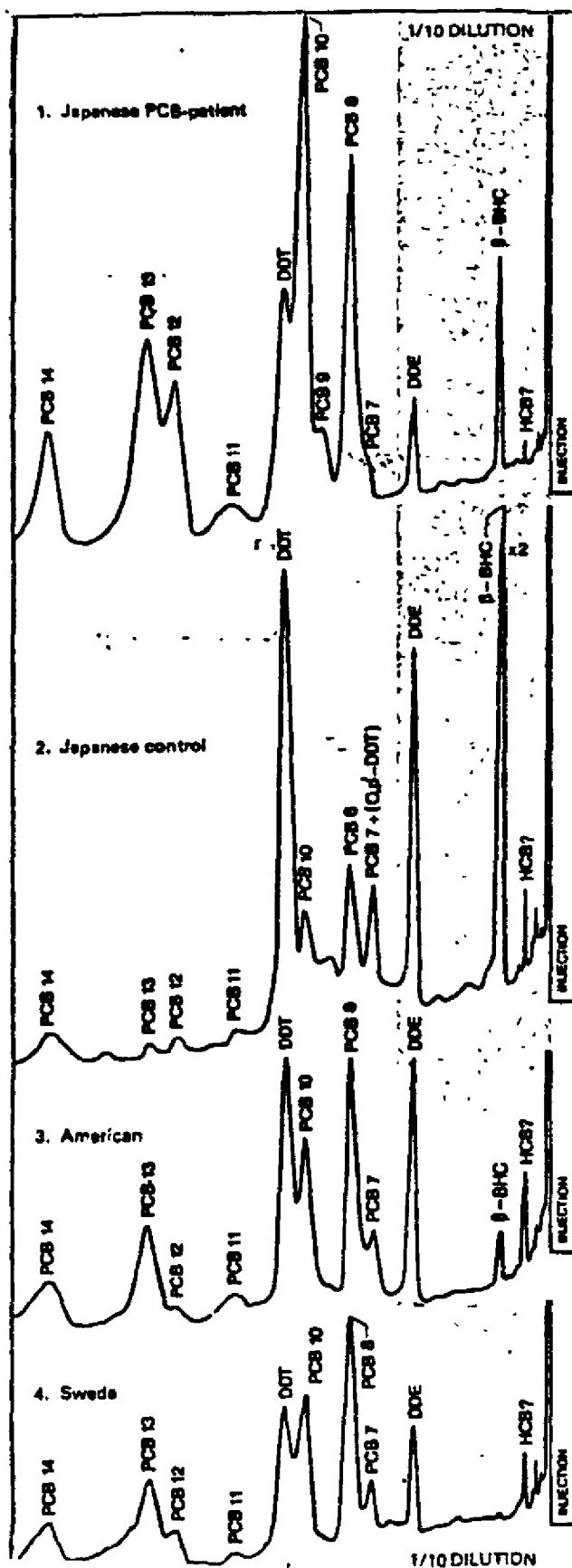


Figure 4. Gas chromatograms of human blood samples, showing levels of PCB, DDT, DDE, β -BHC and HCB(7).

1. Japanese (PCB patient)
2. Japanese (control)
3. American (lived 4 years in Japan, then 4 years in Sweden)
4. Swede

methods have been discovered. In some cases death occurred.

Pregnant women patients are reported to have borne babies with highly pigmented skin. Several babies were born dead. In some cases apparently unaffected women had babies with pigmented skin. Such births occur even today.

PCB IN BLOOD SAMPLES

Recently, I compared the PCB level in the blood of two Japanese suffering from PCB intoxication (contacted via contaminated rice oil in 1968) with that of some Japanese and Swedes who were not known to be especially exposed. The sampling was done in 1972 and the results are shown in Table 2 and Fig. 4.

As expected, the PCB-intoxicated Japanese have more PCB in their blood than the other persons investigated (29 ppb in mean, compared with 10 ppb). Because of the small number of samples in this analysis, it is not possible to determine—on the basis of these results alone—whether or not the high level of residue in the patients is due to their high PCB intake four years ago.

But the striking difference in the blood levels of β -BHC between persons living in Japan and in Sweden can be taken to be a rather clear indication that a persistent chemical actually can remain in the body for at least four years.

The substance β -BHC is isomeric to the insecticide lindane and often occurs as a contaminant in lindane preparations. In all blood samples from Sweden, the levels were close to the detection limit of 0.5 ppb. In blood samples from the Japanese, however, the levels ranged from 68 ppb to 200 ppb. Sample No. 4 is from a Swede who has lived in Japan during the last two years; the β -BHC level is almost as high as in the Japanese samples. Far more interesting, however, is sample No. 11, taken from an American who lived in Japan from 1964 to 1968. This person has been living in Sweden since 1968, but the β -BHC is, nevertheless, at the Japanese level. This clearly

Table 2. β -BHC, DDT, DDE and PCB in human blood. Analyses according to the formic acid/hexane method.

no.				ng/g fresh weight basis			
				β -BHC	DDE	DDT	PCB
1	Swede	♀	Stockholm	0.4	4.5	1.3	6
2	Swede	♀	Stockholm	0.5	6.2	1.6	9
3	Swede	♂	Stockholm	0.6	9.9	3.4	14
4	Swede	♂	Stockholm -1970, Tokyo -72	4.7	10	4.5	11
5	Japanese	♀	Stockholm 1968-70, Tokyo -72	7.8	7.2	3.8	4
6	Japanese	♀	Tokyo	6.8	8.4	3.1	4
7	Japanese	♀	Tokyo	8.3	13	4.1	5
8	Japanese	♂	Southern Japan	20	34	11	8
9	Japanese	♂	Southern Japan (patient)	28	19	7.0	22
10	Japanese	♂	Southern Japan (patient)	43	9.4	5.6	35
11	American	♂	Japan 1964-68	9.4	24	6.0	14
			Sweden 1968-72				

demonstrates that a persistent chemical can actually remain in the body for four years. It follows that this may also be true for PCB.

Another conclusion that can be drawn from this investigation is that the levels of PCB and DDT in the analyzed samples are of the same magnitude, but that the chlorinated insecticide lindane used in Japan contains more β -BHC than that used in Sweden.

It can be seen from Fig. 1 that the relative amounts of the different PCB compounds do not differ in the samples from the two countries.

It has recently been found that low-chlorinated types of PCB can be metabolized at a rather high rate in mammalian organisms. This means that the PCB residue tends to be more similar to high-chlorinated types of PCB (regardless of the chlorination degree of the formulation ingested) because the small amounts of the high-chlorinated homologues also present are unaffected, and thus will eventually dominate.

This may explain why such relatively small amounts of PCB (less than 100 ppm) are found in the bodies of dead Japanese who had been afflicted with PCB intoxication. As the PCB which contaminated the rice oil is said to be of the 30 per cent chlorine type, it may be that all the low-chlorinated types of PCB were metabolized before death occurred, leaving only small amounts of the high-chlorinated ones. If this is true, the PCB levels at the time of intake might have been from ten to 100 times higher than the post mortem analyses indicated.

PCB BANNED BY LAW

Investigations initiated by the National Swedish Environment Protection Board concerning PCB disposal showed that between 10 and 15 tons of the substance were stored

at a firm awaiting safe destruction methods. Some 50 carried 100-200 kg portions of PCB to solid waste pits and one firm dumped the wastes (around 100-200 tons) into the sea. It was only recently that a method of destroying PCB was discovered, as mentioned above.

In January 1972 a Swedish law went into effect prohibiting the use, import, manufacturing and sale of PCB with the permission of the authorities.

PCB probably occurs in more substances than those which are named and forbidden by the new law. An example of this is mentioned above. But the National Swedish Environment Protection Board, the authority responsible for seeing that the law is observed, can in such cases decide that the law shall also cover certain specific products. The possibility of applying the law in this manner is presently being investigated by the Board.

THE MORAL OF THE STORY

The accumulation of PCB in nature was discovered accidentally, as was mercury contamination. What conclusions can be drawn from these findings?

Similar discoveries of the accumulation of other chemicals are quite likely to occur at any time. This should serve to emphasize the need for close cooperation between ecologists, chemists and other scientists.

It is necessary that responsible authorities invest in power and equipment to facilitate an unbiased search for pollutants at an early stage by systematic analysis.

These are the measures that should be taken if the aging and perhaps irremedial effects of a substance are to be discovered before and not after it has entered the environment. This applies especially to substances that accumulate and are highly persistent, as we have learnt from the history of PCB.

EXPERIMENTAL

All solvents used were redistilled. In addition, hexane was also pre-treated with concentrated sulphuric acid and distilled from sodium hydroxide. Methylene chloride was shaken with a 5 per cent solution of potassium carbonate, washed with water and dried with calcium chloride.

Sampling and Extraction: All samplings were conducted at the Museum of Natural History in Stockholm. Samples were stored at -20°C until analysis. Before extraction, the samples were homogenized together with a double amount of anhydrous sodium sulphate by means of an invariable homogenizer. Extraction with hexane was carried out in a Soxhlet apparatus. After four hours the solvent was evaporated, leaving the fat in a mmR, weighed and fixed to the bottom of a pear-shaped flask. Purified nitrogen was blown down to the tube before weighing. Methylene chloride was added in a sufficient amount so that the final solution contained 20 mg fat per 100 μl solution.

Thin layer chromatography: The silicagel G used was washed with methanol (150 ml to 50 g), dried with vacuum and finally activated at 240°C overnight. Microscope slides (15 $\times$ 76 mm) were covered with a slurry of 50 g silicagel in 95 ml water so that the layer was 0.8 mm thick. The plates were stored unactivated in a desiccator above concentrated sulphuric acid and activated at 240°C prior to use.

By means of a Hamilton syringe 100 μl of the above mentioned fat solution in methylene chloride was stripped onto each plate and the plates were developed with methylene chloride.

After air drying, the fat front could be recognized against a lamp as a brown shadow up to a height of about two cm. With a razor blade the silicagel above the fat front was scraped down to an elution tube via a funnel. The tube consisted of a glass tube (10 cm $\times$ 0.8 cm i.d.) onto which was melted a capillary with an inner diameter of 0.15 cm (outer diameter 0.5 cm). Through the capillary was drawn a teflon tube with an outer diameter of 0.15 cm, which was made conical by means of a warm conical glass rod. The chlorinated hydrocarbons were washed out from the silicagel with a mixture of *n*-hexane-diethyl ether (2:3; v/v) until one ml had passed through.

Gas chromatography: As PCB (up to fourteen peaks) and the chlorinated pesticides are extremely difficult to separate with any columns yet developed, at least three different systems were used for the separations, namely one 4 per cent SF 96 methyl silicone, one 6 per cent QF 1 fluor silicone and a third made by mechanically mixing the two mentioned in the proportions two to eight respectively (in the following called the XU-column). *p,p'*-DDE, *a,p'*-DDD and dieldrin could not be separated on a SF 96 column, nor could *p,p'*-DDD and *o,p'*-DDT. On the pure QF 1 column it was possible to fully separate PCB peak 10 (the last major peak) from *p,p'*-DDT, but on the other hand *p,p'*-DDD could not be separated from the PCB peak 8 (the last major peak but one). The XU column gave a full separation of DDE, DDD and DDT for both the ortho-para and para-para isomers.

The allglass column (180 cm, i.d. 0.18 cm) was silanized and filled with the solid support (HMDS-treated 100–120-mesh Gas-Chrom P) covered with the above-mentioned stationary phases. The columns were placed in the gas chromatograph (Varian 204) with an electron capture detector (nitium). The detector and the injector were held at 200°C and the column oven temperature was chosen to give *p,p'*-DDT a retention time of 20 minutes when the solvent retention time was 14 sec. (equal to a mean gas speed of 13 cm/sec.).

Mass spectrometry: The same columns as above were also placed in a LKB 9000 combined gas chromatograph mass spectrometer.

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PCB Poisoning in Japan

by Genayu Umeda

The most serious PCB catastrophe the world has known took place about four years ago in Japan, where thousands of persons are said to have displayed symptoms characteristic of PCB intoxication. Gensyu Umeda describes the disease here.

In March of 1968 a great many cases of a strange, previously unknown disease appeared in the northern and western parts of the Japanese island of Kyushu, particularly in the Fukuoka and Nagasaki prefectures.

Clinical and chemical analyses were performed and in August it was determined that the disease was brought on by some kinds of organic chlorides.

In November it was discovered that the specific cause of the disease was a contaminant in Kanemi Rice Oil, a common food-stuff. The oil was manufactured by the Kanemi Rice Oil Co. Ltd. The contaminant was identified as Kanechlor 400, which is the brand name for a particular PCB (polychlorinated biphenyls). The disease was named "Kanemi Yusho".

There are an estimated 15,000 victims of the disease. However, only 1,081 persons have been officially diagnosed as victims of Kanemi Yusho. Local governments and the central government refuse to officially recognize many of the victims who should, in all fairness, be supported financially by these government bodies.

CLINICAL ANALYSES

Clinical analyses of Kanemi Yusho victims revealed that there are four types of the disease.

Latent type

The patient has no significant physical symptoms and no subjective symptoms of Yusho. Nonetheless, PCB has been detected in patients of this category. For example, when analysis was performed on materials removed surgically, PCB was found. And when women with no obvious symptoms gave birth to babies who displayed typical symptoms of the disease, it became obvious that those women were contaminated. Another example: babies fed with mother's milk displayed pathological symptoms in six months or later.

A baby newly born of a contaminated mother of the latent type displays some characteristic physical symptoms such as hypersecretion of cheese-like or mucoid discharge

from the eyes; disturbance in growth; and pigmentation of the skin and nails and of the mucous membrane of eye and mouth.

Visceral Type

The patient of this type has no characteristic dermatological signs. Instead there are complaints of complicated symptoms as well as physical signs of pathological processes of the viscera. Examples of these are general fatigue, nausea or vomiting, mild jaundice indicating liver injury, colic-like attacks of abdominal pain, diarrhea, productive cough, chronic bronchitis or bronchiectasis, asthmatic attacks, pneumonia. The occurrence of pathological changes of this kind has been demonstrated by x-ray analysis.

In cases of the visceral type, PCB is detected in sputum, blood or material surgically removed, and in many instances there is an increase in the concentration of triglycerides and prebeta-lipoproteins in the blood.

Latent and visceral types are seldom officially recognized as victims of PCB contamination and are seldom diagnosed as Yusho. But these cases are of great significance with regard to the disruption of the environment by PCB and the contamination of the human body by PCB which is liable to occur in the near future.

Manifest type

Many of the cases generally known by the term "Yusho" belong to this type. The characteristic physical signs and subjective symptoms include chloracne, comedo-like eruptions and follicular accentuation as seen in the photo on the opposite page and the photo on page 134. Other symptoms are dark-brownish pigmentation of skin and nails and of the mucous membrane of the eye and mouth; hypersecretion of cheese-like discharge from the eyes; loss of hair; loss of libido; general fatigue; numbness of the extremities or neuralgia-like pain; headache; stomachache; abdominal pain; nausea; vomiting; dizziness; disturbance of higher nervous activities (e.g. forgetfulness), local impairment of blood circulation; vomiting as a result of changes in conditioned reflexes to disturbance from food.

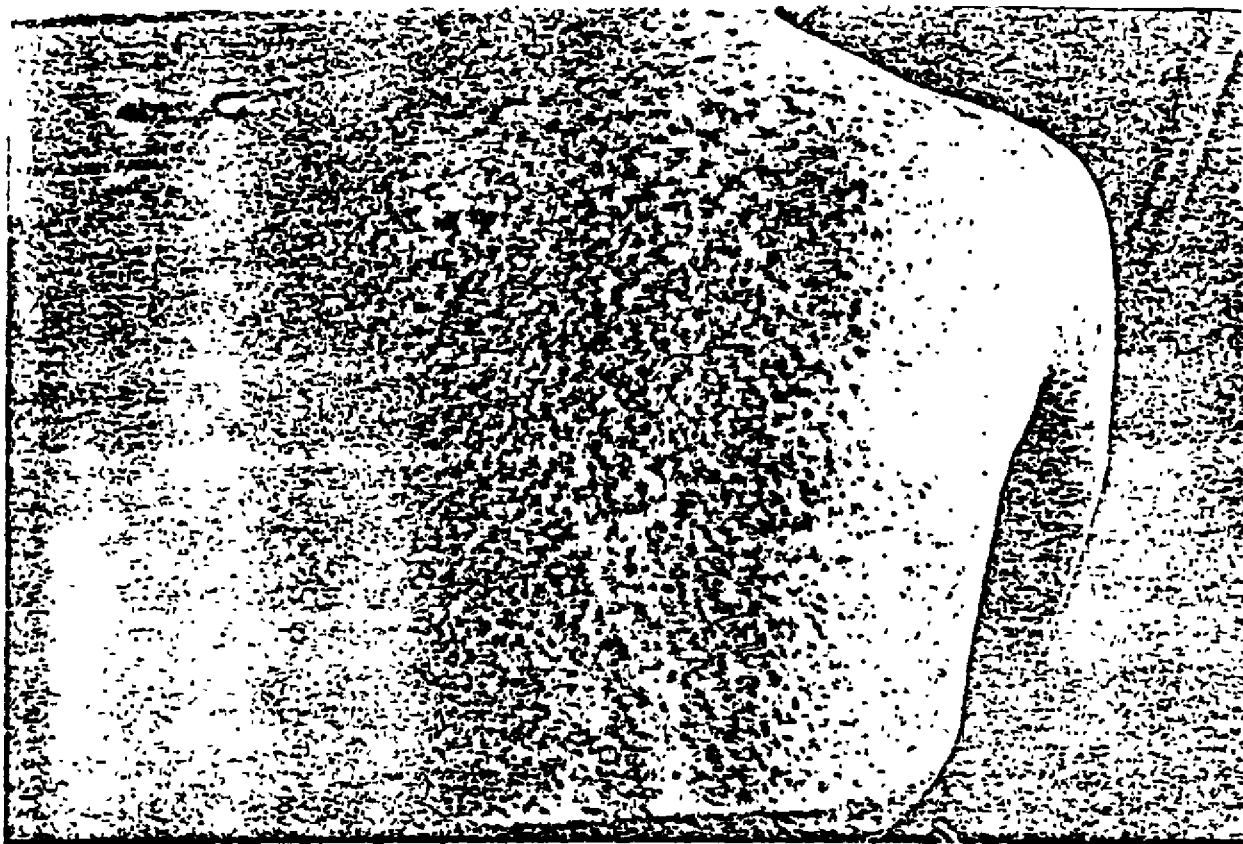
Photos on opposite page and on page 133 show chloracne, comedo-like eruptions and follicular accentuation.

See colour photos on inside back cover of Japanese patients with some of the symptoms of the manifest type of Kanemi Yusho.



AMBIO, AUGUST 1972

MONS 207819



foods; flattening or deformation of nails; deformities of joints and bones; poor development of teeth in children; morphological changes of teeth of a pathological nature. In addition, other pathologically complicated changes have been observed recently.

Although patients suffering from these symptoms are typical Kanemi Yusho cases, many of them have not yet been officially recognized as victims of the disease.

Tardive Type

Some cases of a delayed (tardive) type have been observed. Physical signs and symptoms specific to Yusho are manifested, regardless of the amount of PCB the patient ingested. The symptoms appear one to three years later. The reason for this is not clear at this time (1).

TREATMENT AND PROGNOSIS

Yusho is very difficult to treat and its prognosis is not always benign. Treatments administered in attempts to ameliorate the suffering and complicated symptoms of the patients are generally not successful... with one exception. Patients are slightly ameliorated when human placentas that have been kept in cold storage are administered by injection or transplant. These measures are especially effective with regard to the nonspecific symptom complex or the nonspecific reactions of the organism as a whole.

Acupuncture and moxibustion (used in the Kampō medical approach, a Chinese method of treatment) is recognized to be more effective in dealing with pain such as

headache, stomachache, stiff shoulders, low back pain and pain or numbness of the extremities (2).

Concerning the prognosis, the following symptoms are in my opinion the most serious ones:

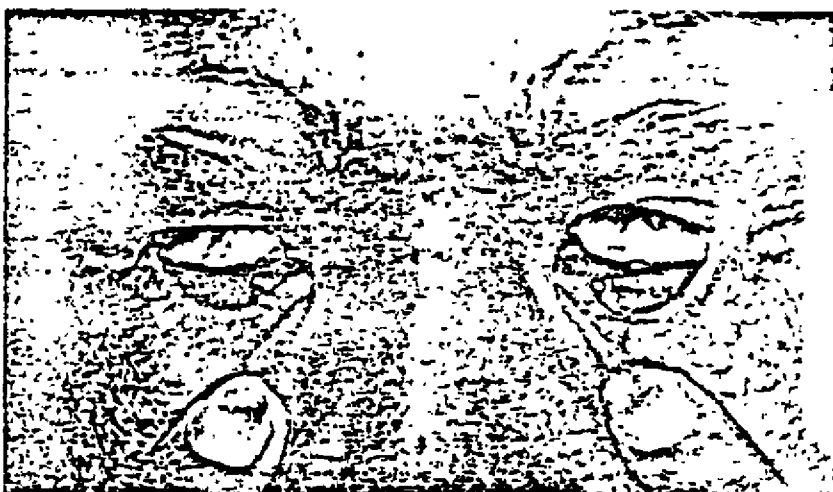
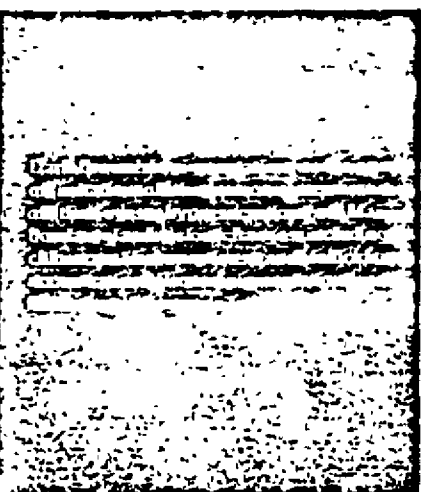
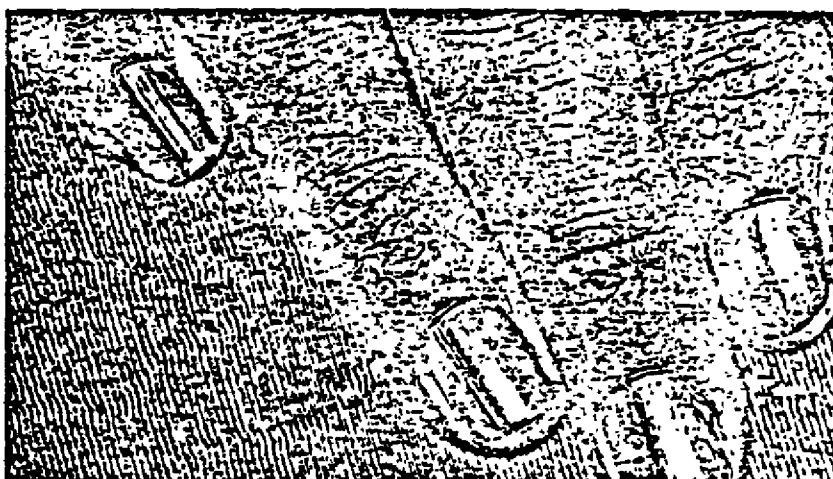
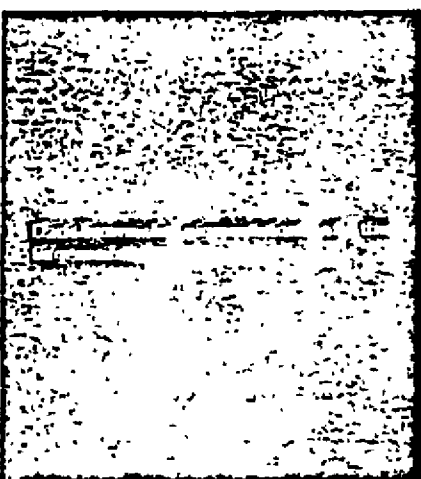
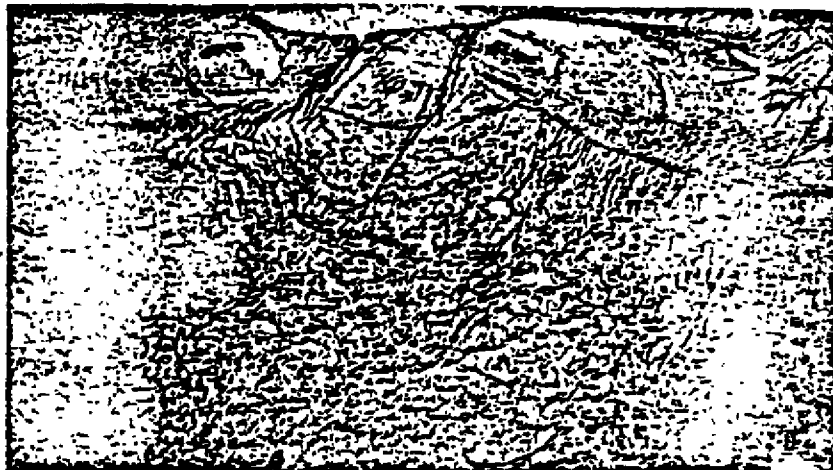
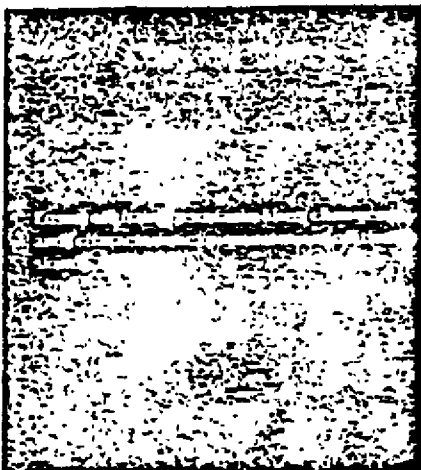
1. Functional disturbance of the higher parts of the central nervous system particularly of the cerebral cortex. This is especially serious in young children, who may display symptoms at birth or during the first years of life.
2. Changes in the cardiovascular system are being observed. This is an important tendency at the present time. The changes may relate both to the function of the heart, as demonstrated by EKG analysis, and to the peripheral circulation. The occurrence of signs of local impairment of blood circulation was already mentioned.
3. A new tendency that has recently been discovered is the deformity of bones; especially deformities of the finger toes, ankles, wrists or vertebrae. The deformities are accompanied by pain.
4. In some cases changes have been observed in the teeth e.g. breakdown, loss or sudden snapping of teeth (3).

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infants - patients with some of the symptoms typical of the infantile
 type of Kanemi-Yusho - a disease caused by the ingestion of PCB
 (see article on page 132)



Original in color