Phytanic acid in Refsum's syndrome

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Refsum (1946) described an heredo-familial degenerative disease of the central and peripheral nervous system which he first called 'heredo-ataxia hemeralopia polyneuritiformis'. Later, because he was unable to prove its identity with the familial heredo-ataxias, the term heredopathia atactica polyneuritiformis was adopted. Numerous reports since (Reese and Bareta, 1950; Clark and Critchley, 1951; Austin, 1956; Fleming, 1957; Ashenhurst, Millar, and Milliken, 1958; Heycock and Wilson, 1958; Gordon and Hudson, 1959) have described cases of this disease to the point where its principal clinical features and the commoner variations are well documented. The basic clinical features are (1) atypical retinitis pigmentosa with concentric constriction of the visual fields; (2) chronic polyneuropathy with symmetrical weakness or paresis of the distal parts of the limbs with absent or diminished deep reflexes; (3) ataxia and nystagmus with other cerebellar signs; (4) increased cerebrospinal fluid protein. Variable additions to this picture include anosmia, abnormal electrocardiographic findings, pupillary abnormalities such as meiosis and sluggish reaction to light, neurogenic deafness, skin changes resembling ichthyosis, and skeletal abnormalities, including pes cavus, epiphyseal dysplasia in the elbow, shoulder and knee joints, and unusual lengthening of metatarsals and metacarpals. Various views on the aetiology and pathogenesis have been expressed. The vasa nervorum have been implicated; the characteristic fibrous proliferation within the nerve trunk was thought to impair conduction by constriction, and, after the most detailed histopathological analysis, Cammermeyer (1956) concluded that the basic pathological change occurred in the myelin sheath.

Klenk and Kahlke (1963) reported the presence of high concentrations of phytanic acid (3, 7, 11, 15 tetramethylhexadecanoic acid) in the blood and post-mortem tissues of a patient with Refsum's disease. Kahlke (1964) has now demonstrated the presence of phytanic acid in the lipids of at least nine cases of Refsum's disease. Clinical details of this original case are given by Richterich, Kahlke, van Mechelen, and Rossi (1963); Richterich, van Mechelen, and

Rossi (1965) and details of the isolation and identification of the storage product are given by Kahlke and Richterich (1965).

The present report presents clinical and post-mortem findings in a case of Refsum's disease in which the presence of phytanic acid has been demonstrated in many tissues, including brain and peripheral nerve.

A second case is presented briefly in which phytanic acid is present in the plasma lipids of a patient with Refsum's disease. These cases confirm Kahlke's findings and are considered to support Cammermeyer's views that the basic lesion is in the myelin sheath. In the sense that the disease can now be regarded as a process of abnormal lipid metabolism, it is to be regarded as one of the lipidoses.

CASE REPORTS

CASE 1 E.M.W. was the illegitimate son of a mother said to be of low grade mentality and a father said to be a diabetic. His early records and the details of his family relationships were lost in the Napier earthquake.

At the age of 9 years his vision began to deteriorate. By the age of 15 years his hearing was grossly defective but the duration of this disability was not recorded. At the age of 19 years he was admitted to Porirua Hospital and at this stage he was quite deaf. The legs were grossly wasted, the muscle power much impaired, and there was gross ataxia. Bilateral pes cavus was present. The hands and arms showed some wasting with intention tremor. He claimed that his disabilities had progressed steadily over the four years since he was 15 years old. In 1957, at the age of 28, his mental state, which had fluctuated somewhat over the years, had deteriorated to the point where he did not speak, smiled foolishly and inconsequently but was not evidently hallucinated or deluded. His behaviour was increasingly antisocial. Physically his health had deteriorated and radiological changes considered to be due to pulmonary tuberculosis were found in both right and left upper lobes. A full neurological examination was made in April 1961 when the patient was 32 years old by Dr. R. W. Hornabrook. This revealed impaired sense of smell on both sides. There was considerable reduction in visual acuity. The left pupil was extremely small (approximately 0.5 mm. in diameter). The pupillary responses to light were sluggish while those to accommodation could not be tested. There was an extreme degree of nystagmus on lateral gaze, vertically

and at rest, so that a 'jelly' nystagmus was clearly visible from the end of the bed. The patient was extremely deaf. There was gross slurring spastic dysarthria. No wasting was visible in the tongue. Gross ataxia and incoordination were found in all coordinated movements of the arms and a similar degree of ataxia was present in the legs. There was profound wasting of the small muscles of both hands, of the lower forearm musculature, and of the muscles of the legs below the knees. There was probably some proximal wasting as well. The deep tendon reflexes in the legs were unobtainable as were the plantar responses. In the arms the biceps reflex and the supinator reflex were probably exaggerated. The triceps reflex was unobtainable. Sensory examination was difficult, but appreciation of pinprick and light touch in the distal segments of the arms and legs appeared to be reduced and there were errors in joint position sense, but this was not severe enough to account for the degree of incoordination witnessed.

Further investigations were planned but the patient's condition deteriorated rapidly at this stage, and he died before anything more could be done.

Post-mortem examination The body showed extreme muscle wasting distal to the knees and elbows. There

was very marked bilateral pes cavus. On dissecting the muscles of the calf they were found to be very pale, soft, and greatly reduced in bulk. The hamstrings were relatively poorly developed but this man was by no means muscular. Their colour and consistency were normal.

Microscopically the peripheral muscles showed extensive atrophic changes of the type associated with peripheral nerve lesions. There were extensive areas of small fibres in contrast to the occasional normal or hypertrophied fibre. The fields of small fibres showed a striking relative increase in the numbers of nuclei but there was also an increase in the size of some nuclei which had bizarre shapes and exhibited hyperchromatism. The changes were most advanced in the peroneal muscles, the hamstrings showed minor changes and the pectoral muscles were not significantly affected. The sciatic nerve was exposed above the popliteal fossa and it stood out as a prominent cord between the relatively small muscles. Its cross-section was found to be twice that of another male cadaver of the same age and general build. Microscopic examination of the sciatic nerve showed that the increase in diameter was attributable in part to the accumulation of an exudate between nerve bundles and beneath the perineurium and in part to an increase in the

FIG. 1

FIG. 1. Transverse section of sciatic nerve to show exudate beneath perineurium. Lipoid droplets appear as clear spaces in paraffin sections (haematoxylin and eosin).

FIG. 2

FIG. 2. Transverse section of sciatic nerve to show thickening of perineurium by condensation and organization in the exudate. Nerve fibres are surrounded by fibrous tissue sheaths (haematoxylin and eosin).

FIG. 3. Myocardium showing fibrosis, mild inflammatory infiltrate, variation in size of myocardial fibres and the nuclei (haematoxylin and eosin).

fibrous tissue surrounding each axon and an increase in thickness of the perineural sheath. The exudate was eosinophilic, weakly P.A.S. positive, and contained some stainable lipid in droplet form (Fig. 1).

The fibrous thickening of the perineurium at least appeared to be related to condensation, lamination, and fibrillary change in the exudate (Fig. 2).

The cross-section of the individual nerve fibres was altered by great reduction in the thickness of the myelin sheath. In many fibres the myelin sheath was no longer distinguishable. The axon was found in most of the fibres and it appeared to be immediately invested by a thickened zone containing longitudinally-running reticulin-staining fibres arranged in an overlapping or imbricated fashion. The changes represented a stage in the development of the more typical 'onion-bulb' appearance. The heart was considerably enlarged by hypertrophy of both right and left ventricles and by dilatation of the left ventricle. The heart muscle was generally pale brown and there were scattered areas of darker appearance distributed haphazardly throughout both ventricles. The valves and coronary vessels were unremarkable. Histologically the myocardium showed widespread diffuse fibrosis. Many large patches of fibrous tissue devoid of muscle fibres were found and these showed at their margins gradual replacement of the myocardial fibres (Fig. 3).

In this marginal zone the muscle fibres were much swollen and frequently contained hyperchromatic irregularly-shaped nuclei. A mild inflammatory infiltrate was also noted in this zone. In the areas of muscle not affected by the fibrous replacement process some nuclear irregularities were found. The nerves of the pericardium appeared unusually prominent and many twiglets showed a clear space beneath the perineurium suggestive of exudate which had dissolved during preparation.

The lungs showed old pleural adhesions and some scarring at the apex of the right upper lobe. There was diffuse pneumonic consolidation of the lower lobe of the left lung.

Gross examination of the brain revealed moderate diffuse meningeal thickening. Microscopically this thickening of pia-arachnoid was associated with gliosis in the outer layers of the cortex. There were ependymal granulations in the ventricular lining of all the ventricles. No abnormalities were found in the cerebellum. The spinal cord was not available for study.

The liver and kidneys were normal in anatomical configuration but were pale in appearance. On histological examination widespread fat vacuolation was noted in the liver cells and in the renal tubular epithelium.

One eye was removed and on section it showed advanced retinal degenerative changes. There was atrophy involving all layers. A single layer of cells apparently represented both the inner and outer nuclear layers and the layer of rods and cones appeared vacuolated, disrupted, and in some places occupied by a collection of spherical bodies. The pigment epithelial layer was tenuous but intact but there was a patchy increase in the amount of pigment in the choroid. The optic nerve was unremarkable. The extrinsic ocular muscles showed no unusual features.

The post-mortem diagnosis was Refsum's syndrome with terminal left lower lobar pneumonia.

Formalin-fixed tissues from this patient were in storage when Kahlke (1963) reported the presence of phytanic acid in a case of Refsum's disease. Gas-liquid chromatography revealed substantial quantities of this material in the tissues from our patient. The technical aspects of this analysis have been reported by Hansen (1965a). The amounts detected are indicated in Table I and each tissue is compared with a control from the same organ although each control comes from a different patient. None of the control patients exhibited neurological disease.

TABLE I

PHYTANIC ACID EXPRESSED AS PERCENTAGE OF TOTAL LIPIDS		
	Patient E.M.W.	Control
Liver	53.6%	Nil
Kidney	44.1%	Nil
Heart muscle	42.2%	Nil
Brain tissue	8.5%	Nil
Sciatic nerve	13.8%	Nil

CASE 2 J.M., an unmarried woman aged 53 years, gives a history of neurological disorder extending over 27 years.

At the present time she is deaf, has loss of visual acuity, nystagmus, ataxia, and loss of deep reflexes and a raised protein level in the cerebrospinal fluid. There is marked symmetrical wasting and weakness of the lower limb muscles, especially distally. No family history of blindness or unusual paralyses is obtainable. A biopsy of an intercostal nerve shows features similar to those found in case 1. There is a thickened perineurium, loss of myelin sheath with formation of a sheath of longitudinally orientated fibrous tissue around each axon. A small amount of exudate giving a P.A.S.-positive reaction is found between the nerve bundles.

Gas-liquid chromatography of the plasma lipids revealed the presence of phytanic acid in a concentration of 7.3% of the total fatty acids. No phytanic acid could be demonstrated in the urine. Further metabolic studies, including radioisotope studies, are in progress on this patient.

DISCUSSION

The original clinical description of Refsum (1946) was based on five cases in two unrelated families. Cammermeyer (1956) described the pathology of Refsum's original cases as identical with the lesions of hypertrophic neuropathy (Dejerine-Sottas disease).

As in case 1 described here, it is common to find aspects of the clinical picture which, taken by themselves, are reminiscent of other neurological disorders. Thus case 1 exhibits pes cavus, ataxia, and myocardial lesions indistinguishable from Friedreich's ataxia. The lesions in the peroneal muscles are identical with the Charcot-Marie-Tooth type of peroneal muscular atrophy. Many of the case reports in the literature also present this mixed type of picture, so that, on the one hand Austin (1956) says, 'the number of forms of this affliction is almost the same as the number of authors who have worked with it', and on the other Spillane (1940) regards the more clear-cut varieties as only 'highlights' in a wider and more obscure background of neuropathy. The discovery of an underlying biochemical disorder in Refsum's syndrome (Klenk and Kahlke, 1963; Richterich *et al.*, 1963; Kahlke, 1963), subsequently confirmed in our case 1, provides a possible means of establishing the fundamental similarity if such exists. However, Baker, Thompson, and Zilkha (1964), in the course of studies on serum fatty acids in multiple sclerosis, used a case of Dejerine-Sottas hypertrophic polyneuritis as a control. Thompson (1964) has reviewed the chromatogram from this patient and has failed to demonstrate any evidence of an abnormal fatty acid. Thus the close resemblance on histopathological grounds is not substantiated biochemically. This is not surprising if one accepts the view expressed by Austin (1956) that the changes in

the peripheral nerves are to be interpreted as the late result of a non-specific secondary tissue reaction. They are regarded as a natural predictable response of the interstitium neither pathognomonic, exotic in kind, nor primary in any constrictive sense.

Refsum (1960) considers that the era has passed when 'neurologists collected rare diseases and syndromes only to describe them like rare stamps'. There is clearly a need to distinguish not only the features which are structural end results but also the pathophysiological or biochemical processes which set in motion the degenerative changes. In the case of Refsum's syndrome the view of Cammermeyer (1956) that the basic lesion was in the structure or composition of the myelin sheath is supported by the finding of an abnormal fatty acid component in the peripheral nerve and brain. This presumably abnormally constituted myelin sheath is undoubtedly progressively destroyed or eliminated and thus is set in motion a tissue response which is dictated by the potential of the local elements, not by the nature of the underlying biochemical disorder. Thus apparently similar structural results may be initiated by a variety of aetiological agents.

Phytanic acid (3, 7, 11, 15 tetramethylhexadecanoic acid) was first discovered by Hansen and Shorland (1952) in butter fat. It has since been found in butter fat by Sonneveld, Begemann, van Beers, Keuning, and Schogt (1962), ox plasma (Lough, 1963), ox perinephric fat (Hansen, 1965b), and sheep fat (Hansen, 1965c) but not in ox spinal cord (Hansen, 1965d). Steinberg, Avigan, Mize, and Baxter (1966) have synthesized phytanic acid from phytol (3, 7, 11, 15 tetramethylhexadec 2-en-1-ol) which is a major constituent of the chlorophyll molecule. Phytanic acid also accumulated in the liver and plasma of rats to which phytol had been fed. This suggests that an exogenous source of phytol such as chlorophyll may serve as a precursor of phytanic acid. The amounts found in butter fat and animal fats are very small but if able to accumulate could be of some significance as a dietary source in humans. In normal animals phytol and its metabolic products are readily oxidized to CO₂ and appreciable storage in normal rats occurred only when the level of phytol in the diet was high. These experimental results would tend to indicate that in Refsum's disease a hereditary metabolic defect, possibly enzymatic, leads to failure to convert phytanic acid, synthesized from phytol, into CO₂ and the accumulated phytanic acid is incorporated into the developing myelin sheath. The myelin so formed is less permanent than normal and in the peripheral nerves leads to gradual loss of myelin sheath and to a process of perineural fibrosis. The evidence so far available suggests that while a somewhat similar

process may be responsible for such syndromes as Dejerine-Sottas hypertrophic neuropathy, the Roussy-Levy syndrome, and possibly peroneal muscular atrophy, it is not an identical biochemical anomaly which causes these diseases. Further studies in case 2 are in progress which may assist in elucidating the biochemical pathways. It is of note that although phytanic acid is present in ox plasma and in butter fat it cannot be demonstrated in the spinal cord of this species. The high levels of Refsum's disease are not found in the normal ox, and the high levels found in the rat in feeding experiments fall very rapidly when phytol is withdrawn from the diet. Our results indicate that phytanic acid is incorporated in both the brain and peripheral nerve tissue in Refsum's syndrome, but no structural changes in the brain are recognized which would suggest extensive myelin loss.

SUMMARY

A full clinical and post-mortem report of a case of Refsum's syndrome is given in which high levels of phytanic acid have been found by gas-liquid chromatography in brain, sciatic nerve, liver, kidney, and heart muscle. Brief clinical details of a second case are given where phytanic acid has been found in the plasma lipids. The literature is reviewed and the significance of this abnormal biochemical constituent in the pathogenesis of Refsum's syndrome is discussed.

Dr. R. W. Hornabrook provided clinical details of case 1 and the Medical Superintendent Porirua Hospital has given permission to publish other details of this case. Dr. J. D. Bergin provided clinical details of case 2. Mr. R. P. Hansen, of the Fats Research Division, D.S.I.R., Wellington, carried out the gas-liquid chromatography. Dr. F. B. Shorland, Director, Fats Research Division D.S.I.R., Wellington, and Dr. I. A. M. Prior, Director Medical Unit, Wellington Hospital, have given advice and encouragement in these studies.

REFERENCES

- Ashenhurst, E. M., Millar, J. H. D., and Milliken, T. G. (1958). Refsum's syndrome affecting a brother and two sisters. *Brit. med. J.*, 2, 415-417.
- Austin, J. H. (1956). Observations on the syndrome of hypertrophic neuritis (The hypertrophic interstitial radiculo-neuropathies). *Medicine (Baltimore)*, 35, 187-237.
- Baker, R. W. R., Thompson, R. H. S., and Zilkha, K. J. (1964). Serum fatty acids in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatr.*, 27, 408-414.
- Cammermeyer, J. (1956). Neuropathological changes in hereditary neuropathies: Manifestation of the syndrome hereditary atactica polyneuritis in the presence of intestinal hypertrophic polyneuropathy. *J. Neuropath. exp. Neurol.*, 15, 340-361.
- Clark, D. B., and Critchley, M. (1951). Hereditary atactica polyneuritis (Refsum's syndrome). *Proc. roy. Soc. Med.*, 44, 689-690.
- Fleming, R. (1957). Refsum's syndrome: an unusual hereditary neuropathy. *Neurology (Minneapolis)*, 7, 476-479.
- Gordon, N., and Hudson, R. E. B. (1959). Refsum's syndrome: Hereditary atactica polyneuritis. A report of three cases, including a study of the cardiac pathology. *Brain*, 82, 41-55.
- Hansen, R. P. (1965a). 3, 7, 11, 15 Tetramethylhexadecanoic acid: its occurrence in the tissues of humans afflicted with Refsum's syndrome. *Biochim. biophys. Acta (Amst.)*, 106, 304-310.
- (1965b). Occurrence of 3, 7, 11, 15 Tetramethylhexadecanoic acid in ox perinephric fat. *Chem. and Industr.*, pp. 303-304.
- (1965c). 3, 7, 11, 15 tetramethylhexadecanoic acid: its occurrence in sheep fat. *N.Z. J. Sci.*, 8, 158-160.
- (1965d). Personal communication.
- , and Shorland, F. B. (1952). The branched-chain fatty acids of butter fat. 2. The isolation of a multi-branched C_{26} saturated fatty acid fraction. *Biochem. J.*, 50, 358-360.
- Heycock, J. B., and Wilson, J. (1958). Diabetes mellitus in a child showing features of Refsum's syndrome. *Arch. Dis. Childh.*, 33, 320-322.
- Kahlke, W. (1963). Über das Vorkommen von 3, 7, 11, 15 Tetramethylhexadecansäure im Blut serum bei Refsum's syndrom. *Klin. Wschr.*, 41, 783-784.
- (1964). Refsum-Syndrom.—Lipoidchemische Untersuchungen bei 9 Fällen. *Ibid.*, 42, 1011-1016.
- , and Richterich, P. (1965). Refsum's disease (Hereditary atactica polyneuritis): an inborn error of lipid metabolism with storage of 3, 7, 11, 15-tetramethyl hexadecanoic acid. II. Isolation and identification of the storage product. *Amer. J. Med.*, 39, 237-241.
- Klenk, E., and Kahlke, W. (1963). Über das Vorkommen der 3, 7, 11, 15-Tetramethyl-hexadecansäure (Phytans äure) im den Cholesterinestern und anderen lipidfractionen der Organe bei einem Krankheitsfall unbekannter Genese (verdacht auf Hereditary atactica polyneuritis (Refsum syndrom)). *Hoppe-Seyler's Z. physiol. Chem.*, 333, 133-139.
- Lough, A. K. (1963). Isolation of 3, 7, 11, 15 tetramethylhexadecanoic acid from ox plasma. *Biochem. J.*, 86, 14P.
- Reese, H., and Bareta, J. (1950). Hereditary atactica polyneuritis. *J. Neuropath. exp. Neurol.*, 9, 385-395.
- Refsum, S. (1946). Hereditary atactica polyneuritis. *Acta psychiat. (Kbh.)*, Suppl. 38.
- (1960). Hereditary atactica polyneuritis reconsideration. *Wld. Neurol.*, 1, pp. 334-347.
- Richterich, R., Kahlke, W., van Mechelen, P., and Rossi, E. (1963). Refsum's syndrome (hereditary atactica polyneuritis) Ein angeborener Defekt im Lipid-Stoffwechsel mit Speicherung von 3, 7, 11, 15 Tetramethyl-Hexadecansäure. *Klin. Wschr.*, 41, 800-801.
- , van Mechelen, P., and Rossi, E. (1965). Refsum's disease (Hereditary atactica polyneuritis): an inborn error of lipid metabolism with storage of 3, 7, 11, 15 tetramethyl hexadecanoic acid. I. Report of a case. *Amer. J. Med.*, 39, 230-236.
- Sonneveld, W., Begemann, P. H., van Beers, G. J., Keuning, R., and Schogt, J. C. M. (1962). 3, 7, 11, 15 tetramethylhexadecanoic acid a constituent of butter fat. *J. Lipid Res.*, 3, 351-355.
- Spillane, J. D. (1940). Familial pes cavus and absent tendon-jerks: its relationship with Friedreich's disease and peroneal muscular atrophy. *Brain*, 63, 275-290.
- Steinberg, D., Avigan, J., Mize, C., and Baxter, J. (1966). Phytanic acid formation and accumulation in Phytol fed rats. *Biochem. biophys. Res. Commun.*, in the press.
- Thompson, R. H. S. (1964). Personal communication.