state of augmented iron absorption, known to occur during the treatment of pernicious anaemia with vitamin B₁₂ (Pirzio-Biroli *et al.*, 1958), which we feel might explain the high incidence of beeturia in the pernicious-anaemia patients.

Some of the 14% incidence of beeturia in normal subjects could be attributed to the fluctuating nature of iron absorption in normal people. In this respect it may be relevant that when some of the positives in the control group were retested the result was negative. Moore (1955) stresses that the regulation of iron absorption by the gut is not absolutely related to tissue needs. Clearly the problem is much more complex than the simple genetic hypothesis of Allison and McWhirter (1956), whose results were variable, whose conclusions have been challenged (Penrose, 1957), and whose views, even if correct, relate only to the incidence of the phenomenon in normal people and not to its mechanism. Other theoretical considerations which might be relevant in relating iron to the transport of betacyanin include the effect of iron deficiency on the function of cytochrome enzymes, or the reciprocal chelating properties of iron and betacyanin.

Experimental studies have been carried out to test other factors which might influence betanin absorption. Full body-weight doses of histamine, large doses of ascorbic acid, and 0.1N hydrochloric acid have not significantly affected repeat tests which were negative first time. The giving of food introduces so many variables that we have avoided this at present.

The possibility of a relationship between idiopathic enteropathy and beeturia is still undecided. The numbers do not permit statistical proof of an association, but the incidence of three positive tests in nine patients with

jejunal atrophy and no significant anaemia is suspiciously high, considering how strongly positive two of the tests were.

Further work on the metabolism of betanin is in progress, and we hope in the future to be able to give a more definitive account of this rather neglected clinical phenomenon.

Summary

Beeturia occurs in 13.8% of the normal population. It is not a universal phenomenon as is popularly supposed, nor is it limited to patients with food allergy as others claim.

The incidence of beeturia in patients with iron-deficiency anaemia is 48.6% and in iron-deficient subjects not previously treated with oral iron it is 80%. The influence of oral iron therapy on the phenomenon suggests that the apoferritin carrier mechanism may play some part in its occurrence.

There is an unsubstantiated possibility that beeturia may occur in certain kinds of "idiopathic enteropathy."

We are grateful to Professor E. M. McGirr for reading this paper and allowing us to study patients under his care.

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BLOOD PYRUVIC ACID IN THALIDOMIDE NEUROPATHY

BY

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Prior to the recognition of the teratogenic properties of thalidomide, numerous cases of peripheral neuropathy had been reported in adults after its use (Fullerton and Kremer, 1961; Burley, 1961). But its mode of action in causing the peripheral neuropathy was uncertain. Antagonism of the activity of vitamins of the B-complex was suggested by Robertson (1962), whilst an impairment of glucose absorption was reported by Meade and Rosalki (1961), although Kremer and Fullerton (1961) could find little evidence for this. Furthermore, any interference of glucose metabolism seemed unlikely in view of the finding of normal quantities of pyruvic acid in the blood (Fullerton and Kremer, 1961).

However, the standard methods in current use for estimating pyruvic acid, such as that of Friedemann and Haugen (1943), are unsatisfactory in so far as they are not specific and measure in addition other α -keto acids present in the blood; indeed, pyruvic acid may account for only 40-75% of the total (Cavallini, Frontali, and Toschi, 1949; El Hawary and Thompson, 1953), so that minor variations in its concentration may not be detected. Chromatography of the phenylhydrazine derivatives of keto acids (Cavallini *et al.*, 1949) allows of the separation of pyruvic acid from the other keto acids and enables its accurate determination (El Hawary and Thompson, 1953), so that lesser degrees of abnormality of pyruvate metabolism may thereby be detected (McArdle, 1957). This paper describes the results

of estimations of blood pyruvic acid by chromatography in three patients with thalidomide neuropathy.

Case 1

A retired nurse aged 66 presented with a 10-months history of aching pains in the feet, which progressively became worse and spread to involve the thighs. She experienced difficulty in walking owing to dead feelings in the legs and noticed stiffness and slight swelling of the feet. Three months before she was seen she developed paraesthesiae and numbness in the feet and some weeks later similar symptoms in the hands. Dietetic inquiry did not suggest any deficiency of vitamins in the diet, nor was there any history of an excessive intake of alcohol. The patient had been taking thalidomide, in a nightly dose of 200 mg., for some months before the onset of symptoms.

Her nutrition appeared satisfactory; the blood-pressure was 160/90 mm. Hg and there was slight oedema of the ankles, but no elevation of the jugular venous pressure; the general examination was otherwise normal. In the motor system the only abnormalities found were sluggish knee-jerks and absent ankle-jerks; the plantar responses were flexor. There was a patchy impairment in the appreciation of light touch and pin-prick in both legs below the level of the mid-calf, but no sensory disturbance could be detected in the arms.

Investigations.—The haemoglobin was 14.7 g./100 ml., and the red blood cells and their indices were normal. A fractional test meal showed the presence of normal quantities of free

acid, and the glucose-tolerance curve was normal, the rise of blood sugar being 40 mg./100 ml. The blood Wassermann reaction was negative and the liver-function tests showed nothing abnormal. The cerebrospinal fluid contained 58 mg. of protein/100 ml., and the globulin was slightly raised, but the Lange colloidal gold curve was normal. The electrocardiogram was normal and chest x-ray examination showed a normal-sized heart.

Case 2

A married woman aged 67 first noticed numbness in the feet 18 months before she was seen. Subsequently she developed paraesthesiae in the feet and aching pains in the calves; these symptoms progressively worsened, so that finally walking became difficult. Twelve months before she was seen she felt pains in the tips of the fingers and beneath the nails, and later numbness and paraesthesiae and a feeling of stiffness in the fingers. She had been taking thalidomide, 100 mg. nightly, for six months before the onset of her symptoms; she did not drink and there was no suggestion of any vitamin deficiency in the diet. In the past she had been found to be mildly hypertensive and to have a hiatus hernia.

She appeared well nourished; the blood-pressure was initially 170/100, but later settled to 140/85 with rest in bed; the heart was normal in size and there was no evidence of congestive cardiac failure; the general examination was otherwise normal. There were no abnormalities in the motor system apart from absent ankle-jerks; the plantar responses were flexor. The appreciation of light touch, pin-prick, and vibration sense were impaired in the feet and legs up to the level of the mid-calf, and in the hands as far as the wrists. The appreciation of temperature, position sense, and deep pain were normal.

Investigations.—The haemoglobin was 14.2 g./100 ml., and the appearance of the red blood cells and their indices were normal. A fractional test meal did not reveal the presence of any free acid, but the effect of histamine was not determined; a Schilling test showed a normal rate of absorption of vitamin B₁₂. The glucose-tolerance curve was normal, the rise of blood sugar being 40 mg./100 ml. The blood Wassermann reaction was negative. The electrocardiogram showed mild ischaemic changes, but chest x-ray examination showed a normal-sized heart.

Case 3

A professor of music aged 63 presented with a history of paraesthesiae in the feet and hands for six to eight months; these slowly became worse, but she did not experience any numbness in the limbs. She also complained of pain in the upper thighs, but this was thought to result from osteoarthritis of the hips. Dietetic inquiry did not suggest any vitamin deficiency in the diet and she drank only a little alcohol. She had taken thalidomide intermittently, in a nightly dose of 50–100 mg., for two years before the onset of symptoms. She had a long past history of backache and symptoms attributable to osteoarthritis of the hips; 22 years previously she had received a course of gold injections for arthritis.

On general examination she was overweight, the blood-pressure was 140/80, the heart was normal, and there was no evidence of congestive cardiac failure. Apart from limitation of movement at both hips, there were no other abnormalities. The motor system was normal, all the deep tendon reflexes were present and the plantar responses were flexor. There was impairment of appreciation of light touch and pin-prick on the sides of the fingers, while in the lower limbs there was impairment of light touch over the feet and a patchy impairment in the legs up to the level of the knees, although sensation to pin-prick was normal; the calves were a little tender to the touch.

Investigations.—The haemoglobin was 13.3 g./100 ml., and the appearance of the red blood cells and their indices were normal. A fractional test meal showed the presence of normal quantities of free acid, and the glucose-tolerance curve was normal, the rise of blood sugar being 60 mg./100 ml. The blood Wassermann reaction was negative and the liver-function tests were normal.

Estimation of Blood Pyruvic Acid

Methods.—Pyruvic acid was measured by a modification of the method of Cavallini *et al.* (1949); the 2:4-dinitro-phenylhydrazine derivative of pyruvic acid was separated from the other α -keto acid hydrazines by descending chromatography on buffered filter paper (pH 8.6), using a solvent system of *n*-butanol-ethanol-water (70:10:20 by volume) as described in detail elsewhere (Buckle, 1963). Venous blood was collected after a 14-hour overnight fast and further samples were taken one and one and a half hours after giving 100 g. of glucose by mouth (Williams, Mason, Power, and Wilder, 1943; Joiner, McArdle, and Thompson, 1950). The subjects were kept at rest in bed for the duration of the test and the blood was collected with a minimum of venous stasis and without any clenching of the hand.

Normal Subjects.—The mean fasting level of pyruvic acid in 15 normal subjects and 20 hospital patients not suffering from any known nutritional, metabolic, or endocrine disorder was found to be 275 (± 34) μ g./100 ml., and compares with similar values of 230–340 μ g./100 ml. reported by Cavallini *et al.* (1949), Walshe (1955), Hennes, Wajchenberg, Fajans, and Conn (1957), and Hockaday (1961), but is lower than the mean values of 350–440 μ g./100 ml. observed by Smith and Taylor (1955), Biserte and Dassonville (1956), and El Hawary and Thompson (1953). In the controls pyruvic acid rose to mean values of 372 (± 60) and 352 (± 71) μ g./100 ml. at one and one and a half hours respectively after glucose. For subsequent comparisons, values greater than these plus twice their S.D. have been taken as being abnormally elevated.

Patients with Neuropathy.—**Case 1:** The fasting blood pyruvate was 298 μ g./100 ml. and rose abnormally to values of 534 and 523 μ g./100 ml. at one and one and a half hours after glucose (see Chart). On another occasion pyruvic acid was measured by the standard method of Friedemann and Haugen; the fasting level was 1,160 μ g./100 ml. (normal $<1,000$ μ g./100 ml.), and rose to 1,200 μ g./100 ml. one hour after glucose (normal $<1,240$

Pyruvic-acid-tolerance tests. 50 g. of glucose was given orally at time 0 and 1/2 hour. ○—○ initial pyruvate levels. ×—× pyruvate levels after treatment with thiamine. The mean normal values with their standard deviations are given in each case.

$\mu\text{g.}/100\text{ ml.}$). Case 2: The fasting blood pyruvate was $335\text{ }\mu\text{g.}/100\text{ ml.}$ and rose to values of 532 and $553\text{ }\mu\text{g.}/100\text{ ml.}$ at one and one and a half hours after glucose. A repeat pyruvate-tolerance test following seven days' treatment with intramuscular thiamine hydrochloride (30 mg./day) showed a fall in all the pyruvate levels to normal (see Chart). Case 3: The fasting pyruvate was $308\text{ }\mu\text{g.}/100\text{ ml.}$ and after glucose the levels rose to values of 1,183 and $776\text{ }\mu\text{g.}/100\text{ ml.}$ A repeat pyruvate-tolerance test following three weeks' treatment with thiamine hydrochloride by mouth (75 mg./day) showed a return of the values to normal (see Chart).

Treatment with thiamine has been continued in these patients, but there has been no change in the manifestations of the neuropathy.

Discussion

The chromatographic separation and individual measurement of pyruvic acid demonstrated an abnormality in each patient. The clinical features were similar to those of previously reported cases due to thalidomide (Fullerton and Kremer, 1961), and no other cause could be found for the neuropathy. Elevation of blood pyruvate may also occur in certain diseases not necessarily associated with peripheral neuropathy, such as thyrotoxicosis, diabetes mellitus, liver disease, or severe cardiac failure (Joiner *et al.*, 1950; Amatuzio, Shrifter, Stutzman, and Nesbitt, 1952; Walshe, 1955; Hennes *et al.*, 1957), but no such disorder was present in these patients. The abnormality of pyruvate metabolism appeared, therefore, to be associated with the neuropathy following use of thalidomide.

Pyruvic acid is formed as an intermediary during glycolysis and is normally decarboxylated by pyruvate oxidase. Impairment in the activity of the latter may result from a deficiency of its coenzyme thiamine, or from an interaction between its SH groups and a number of toxic agents (Peters, 1936; Thompson and King, 1957); in either case pyruvate accumulates and a peripheral neuropathy may occur (Thompson and Johnson, 1935; Peters, 1936; Platt and Lu, 1936; Joiner *et al.*, 1950; Thompson and King, 1957). But an accumulation of pyruvate is not necessarily indicative of an impairment of pyruvate oxidase, for interference with subsequent steps in the breakdown of pyruvate may also lead to its accumulation in the blood (El Hawary, 1955). However, the observation that blood pyruvate returned to normal in both the patients treated with thiamine suggested that in thalidomide neuropathy there is a disturbance of some thiamine-dependent enzyme system. Furthermore, pyruvate oxidase is particularly susceptible to a deficiency of thiamine (Peters, 1936; Thompson and King, 1957).

Thalidomide itself, or one of its degradation products, might compete with thiamine for attachment to the protein component of the enzyme system, or might combine direct with thiamine, so inactivating it. However, if such a conditioned deficiency of the vitamin was the actual cause of thalidomide neuropathy it might be anticipated that its clinical features should closely resemble those of classical thiamine deficiency. In fact, thalidomide causes predominantly sensory manifestations, while thiamine deficiency usually gives rise to a mixed motor and sensory neuropathy. Moreover, continued treatment with thiamine was without effect, whereas in thiamine deficiency the subjective symptoms usually improve, even though the abnormal neurological signs may be slow to resolve (Platt and Lu, 1936; Williams *et al.*, 1943).

Pyruvic acid is not toxic in itself (Platt and Lu, 1939) and its accumulation is not the cause of peripheral neuro-

pathy, but is merely an associated feature of the common underlying biochemical abnormality. An accumulation of pyruvate might occur if thalidomide interfered with the alternative pathways of carbohydrate metabolism. Disproportionate amounts of glucose would then need to be broken down by glycolysis and pyruvate might accumulate if the quantity produced exceeded the normal capacity of pyruvate oxidase. Consequently, treatment with large doses of thiamine might enable the additional pyruvate to be decarboxylated, without necessarily affecting the primary biochemical abnormality responsible for the neuropathy.

It has been possible to investigate only three patients, for further cases of thalidomide neuropathy are unlikely to occur, but abnormal pyruvate levels were detected in each. These findings, if confirmed by studies in experimental animals, provide direct biochemical evidence for a metabolic abnormality that may be partly responsible for the toxic effects of thalidomide.

Summary

The individual measurement of blood pyruvic acid in three patients with thalidomide neuropathy was performed by chromatography of its phenylhydrazine derivative. An abnormality of pyruvate metabolism was detected in each, and in two patients treated with thiamine the pyruvate levels fell to normal.

The significance of these findings as evidence for a metabolic disturbance induced by thalidomide is discussed.

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The Australian Prime Minister, Sir Robert Menzies, opened the Western Pacific Regional Conference of the World Health Organization in Port Moresby on September 5. Thirteen countries were taking part. Reviewing the progress of health work in Papua-New Guinea, Sir Robert said that after the last war there were only two hospitals left standing in the Territory. In June this year there were 100 Government hospitals and a large number of mission hospitals, nearly 150 doctors, and 4,400 other medical personnel. In the last 10 years £A.33m. had been spent on health services in Papua and New Guinea.