Summary

Twelve patients with Addisonian pernicious anaemia were treated with daily oral doses of vitamin-B₁₂-peptide complex for up to four years. While a dose of 100 µg. of the peptide complex daily was sufficient to maintain normal haemoglobin values it was insufficient to maintain the serum-vitamin-B₁₂ level.

A daily dose of 300 µg. of the peptide complex, however, was adequate, and it is suggested that such a method of therapy is safe and is of particular value in people who dislike injections.

We are grateful to Dr. William Phillips for allowing us to study cases under his care; to Mr. D. A. Greenman for considerable technical assistance; and to Distillers Co.

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ORAL TREATMENT OF SUBACUTE COMBINED DEGENERATION OF SPINAL CORD

BY

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From time to time during the past four years we have reported results obtained from the oral treatment of pernicious anaemia, using vitamin-B₁₂-peptide complexes (Heathcote and Mooney, 1958a, 1958b, 1961; Mooney and Heathcote, 1960, 1961a). With the exception of one patient mentioned in our first communication, all were uncomplicated examples of the disease. The present paper describes the results in 11 cases that showed, in addition to pernicious anaemia, evidence of degeneration in the nervous system.

The theory upon which the treatment is based has been fully described in our previous communications. It rests mainly upon our belief that pernicious anaemia is due to impaired gastric proteolysis of bound forms of vitamin B₁₂, and that intrinsic factor—as currently understood—does not exist.

The diagnosis of megaloblastic anaemia was confirmed in each case by sternal-marrow examination, histamine-alcohol test meal, and by examination of the peripheral blood. Haematological progress was assessed by packed cell volume (P.C.V.) readings performed on the Hawksley microcentrifuge, and by haemoglobin estimation (100% Hb = 14.8 g./100 ml.).

The serum-vitamin-B₁₂ concentration was estimated by the acidimetric titration of 72-hour cultures of *Lactobacillus leichmanii* using "Difco" vitamin-B₁₂ assay broth. All sera were cyanide-treated before assay.

All except Case 7—which in the early stages of treatment received a vitamin-B₁₂-peptide derived from a streptomyces source—have been treated with complexes derived from a propionibacterium. The ratio of associated peptide has varied slightly in the different complexes but has never exceeded 7.1:1.

The doses of the different batches of the peptide complex are given in terms of the cobalamin content alone as estimated spectrophotometrically at 550 mµ. Where crystalline vitamin B₁₂ has been used as such it is mentioned specifically in the text.

Case 7

A woman aged 52 was treated for pernicious anaemia with one tablet of "bifacton" twice daily for 16 months.

At the end of that time, despite the achievement and maintenance of a satisfactory blood-picture, she noticed that her thighs and legs were becoming stiff and difficult to move. Numbness of the feet also appeared, but the hands were unaffected. Vibration sense was lost over the ankles; knee- and ankle-jerks were diminished, and the plantar responses were equivocal. The red-cell count was 4,300,000/c.mm. and the Hb 94% at this time. Early subacute combined degeneration of the spinal cord was diagnosed, and she was treated initially with a streptomyces complex. For three weeks she received 300 µg. daily, and thereafter 200 µg./day for eight weeks. Oral treatment was then suspended owing to shortage of experimental material, and parenteral crystalline vitamin B₁₂ (1,000 µg.) was given every week for a whole year. Oral treatment was then restarted, but with a propionibacterium vitamin-B₁₂-peptide complex at a daily dose of 300 µg. This has remained unchanged up to the time of writing (234th week).

During the first period of oral treatment the stiffness in the lower limbs lessened considerably and her walking improved. Numbness in the feet diminished until only the toes were affected. Vibration sense returned to the left ankle, and also to the right external malleolus during this period, but there was no detectable change in the deep reflexes. During parenteral therapy the improvement in walking continued at about the same rate, but no further change appeared in the objective signs.

The second period of oral treatment began on the 440th day, and by the 666th day vibration sense had returned to the remaining (right internal) malleolus. By this time the only residual symptom was a slight tingling in the feet which came on when she was tired. She returned to work as an office cleaner on the 600th day.

At the time of writing the Hb was 92% and P.C.V. 42%. Her serum-vitamin-B₁₂ level was 240 µµg./ml. The knee- and ankle-jerks were normal but the plantar responses remained equivocal.

Case 34

A man aged 60 had a haemoglobin of 69% and a P.C.V. of 27%. The serum vitamin B₁₂ was 30 µµg./ml. His presenting symptoms were weakness, asthenia, and coldness and numbness of the legs and pelvis, together with severe lower abdominal girdle-pains on defaecation. His knees and ankles were stiff and the leg reflexes were absent. Plantar responses were flexor, but vibration sense was absent from the knees downwards. The gait was extremely

unsteady and he was unable to keep his balance without assistance even with the eyes open. The symptoms had been present for six months, but the upper limbs were not affected. The regime for treatment was:

Days	Daily Dosage (in Terms of $\mu\text{g. Vitamin B}_{12}$)
1 to 45	300
46 " 667	400
668 " 725	300
726 " 1,017	200
1,018 " 1,109	100

Average daily dose, 280 $\mu\text{g.}$

A brisk haematological response occurred, and by the 14th day the Hb was 92% and the P.C.V. 43%. The reticulocyte crisis was maximal (12.6%) on the fifth day.

On the fourth day of treatment his legs began to feel warm, although the serum-vitamin-B₁₂ level was still only 40 $\mu\text{g./ml.}$ After a fortnight the abdominal girdle pains disappeared, and the numbness around the pelvis had lessened. His sense of balance also improved, and after four weeks he could walk about twenty paces without assistance—although the gait was broad-based and rather ataxic. Improvement continued, and after six months normal sensation had returned to the pelvis and upper thirds of the thighs, and he could stroll for about half a mile. No alteration was observed, however, in either vibration sense or reflexes during this period.

After 12 months he returned to work as a "checker"; this necessitated his clambering into loaded vehicles to inspect the contents. He still had a partial residual numbness distal to the mid-thighs, but was walking well and his gait was virtually normal. The first objective change did not occur until after two years, when vibration sense became faintly discernible, first over the left and then—three months later—over the right tibia. Normal sensation has now returned to the thighs, and although the paraesthesia in legs and in feet have not disappeared, they have diminished. Knee and ankle reflexes are absent, and there is still a slight sway when he stands with feet together and eyes closed. At the time of writing his Hb is 101%, P.C.V. 48%, and serum vitamin B₁₂ 500 $\mu\text{g./ml.}$

Case 40

A housewife aged 41 was mentally confused when admitted to hospital, and she remembers nothing of her first 14 days in hospital. She was severely anaemic (Hb 32%; P.C.V. 13%), and pernicious anaemia was diagnosed. Before treatment was started, however, a lobar pneumonia developed, and it was decided, under the circumstances, to treat the anaemia with parenteral vitamin B₁₂ (1,000 $\mu\text{g.}$ every three days). In addition she received 2 pints (1,140 ml.) of blood and parenteral penicillin (0.5 mega-unit eight-hourly). The pneumonia responded quickly and satisfactorily, and a good haematological response was obtained. The Hb was 84% and the P.C.V. 43% on the 39th day. Her mental state also improved rapidly, and on the 14th day she had recovered sufficiently to allow a complete history to be taken. It then became apparent that for 10 months before admission, in addition to anaemia, she had developed a progressive ataxia. This had worsened during the month prior to admission to the point where she had been unable either to stand or to walk without assistance. An increasingly severe numbness had also appeared, which ultimately involved the entire lower limbs, lower half of the abdomen, forearms, hands, and fingers. She was unable to write, knit, or use a knife and fork. Vibration sense was lost over the anterior/superior iliac spines and below. Knee- and ankle-jerks were exaggerated and plantar responses extensor. Co-ordination of leg movements and sensation in the joints of the big toes were both extremely poor. She was unable to recognize objects placed in her hands.

During parenteral therapy, in addition to the mental improvement noted above, the numbness in her lower limbs and hands diminished, and the extremities became less cold.

No objective changes were detected, however, and she was still unable to walk without assistance.

On the 40th day parenteral treatment was stopped, and instead 400 $\mu\text{g.}$ of vitamin-B₁₂-peptide complex was given daily. On the 67th day this was reduced to 300 $\mu\text{g.}$, on the 123rd day to 200 $\mu\text{g.}$, and on the 594th day to 100 $\mu\text{g.}$ a day. This dosage remained unchanged up to the time of writing (923rd day), and the average daily dose was 152 $\mu\text{g.}$

During the first three months on oral treatment the abdominal paraesthesia disappeared and the ataxia improved sufficiently to allow her to walk about 100 yards (90 metres) without assistance. Co-ordination of her hand movements improved to the point where she could manage to peel an apple. After six months she could stand with feet together and eyes closed for 15 seconds; she could write her name and knit for about 10 minutes. By nine months normal sensation had returned to the hands and thighs. Considerable numbness was still present in the legs but was less in the feet. At 12 months the gait had returned to normal and she could walk about eight miles (13 km.). She could carry out household duties without assistance and could knit for two hours. By 18 months the only residual subjective symptom was a heaviness and coldness around the knees, and this disappeared three months later. She now rides a bicycle, drives a car, dances, and she works as a supervisor in a factory canteen. Knee- and ankle-jerks remain brisk, but vibration sense has now returned to the knees and tibiae. The plantar responses are "doubtful." At the time of writing the Hb is 97%, P.C.V. 47%, and serum-vitamin-B₁₂ level 360 $\mu\text{g./ml.}$

Case 47

The patient was a housewife aged 64. Initially her Hb was 56%, her P.C.V. 21%, and her serum-vitamin-B₁₂ level 64 $\mu\text{g./ml.}$ In addition to anaemia, for the past 10 months she had suffered increasing difficulty in using her hands. There had also been a progressive loss of sensation, both superficial and deep, in the forearms, hands, and fingers, her hands having been burned occasionally by the hot handles of pans without awareness. She could not write, knit, or use a knife and fork. Her walking was extremely unsteady, and she held on to furniture as she moved around the house. She was just able to stand, unaided, with eyes open and feet apart. Sensory loss was marked over the entire lower limbs and the lower half of the abdomen. When washing her face she had to turn round and rest her back against the wash-bowl to prevent herself from falling through lack of balance.

Vibration sense was absent over elbows, anterior superior iliac spines, and all distal points. Joint-sense of the big toes and co-ordination of the hands and of the leg movements were severely affected. Both ankle-jerks and the left knee-jerk were absent; the right knee-jerk was just discernible. The left plantar reflex was flexor, the right was equivocal.

Vitamin-B₁₂-peptide complex (300 $\mu\text{g.}$) was given daily for 10 days, and it was then increased to 400 $\mu\text{g./day.}$ A reduction to 300 $\mu\text{g.}$ daily was made on the 202nd day and to 200 $\mu\text{g.}$ daily on the 408th day. The dosage has remained unaltered up to the 777th day. The average daily dose was 276 $\mu\text{g.}$

During the first six months normal sensation returned to the forearms and palms of the hand. The fingers also became rather less clumsy and the numbness diminished. Walking improved during the same period, and she was able to walk up to 400 yards (365 metres) at the end of that time. Her balance became steadier, and she was able to stand with her feet together and her eyes closed for 15 seconds without overbalancing. Numbness disappeared from the abdomen and legs, but persisted to a lessened degree in the thighs and feet. The absent knee- and ankle-jerks returned and the right plantar response became "doubtful flexor" during the same period.

During the next six months vibration sense reappeared over the elbows, wrists, and anterior superior iliac spines. Co-ordination of hand movements continued to improve sufficiently to allow a little knitting for periods of about half an hour. (After this time the needles tended to "fall from her hands.") Normal sensation returned to both thighs quite suddenly at 16 months. After two years' treatment she was able to walk about a mile (1.6 km.). Romberg's test showed slight sway after 30 seconds. Some residual numbness was present in the fingers, but this was insufficient to prevent her from sewing. Partial numbness was still present in the toes and distal halves of the feet. Vibration sense and plantar responses showed no further change.

Case 55

A man aged 72 was comatose on admission to hospital. The Hb level was 25% ; the P.C.V. 10% ; the serum-vitamin-B₁₂ level 42 $\mu\text{g.}/\text{ml.}$; and the blood urea 300 $\text{mg.}/100 \text{ ml.}$ Two pints (1,140 ml.) of blood were given and 300 $\mu\text{g.}$ of vitamin-B₁₂-peptide complex daily for seven days. Treatment was then suspended, as an attempt was being made to discover the maximum response obtainable from this dosage. By the 42nd day the blood urea had fallen to 70 $\text{mg.}/100 \text{ ml.}$, and, although the Hb had risen to only 66%, the P.C.V. had reached 35%, revealing that red-cell production was outstripping that of the haemoglobin, producing a temporary iron-deficiency state. On the 43rd day, when the first attempt at walking was made, he was severely ataxic. Inquiry then revealed that for four months prior to admission he had noticed an increasing unsteadiness of gait, and had been compelled to hold on to furniture when he walked. Stiffness of the lower limbs had also appeared, and there was considerable tightness of the lower part of the abdomen. Numbness had also developed in the entire lower limbs and in the fingers distal to the proximal interphalangeal joints. Knee- and ankle-jerks were absent. The right plantar response was "indefinite" ; the left flexor. Postural sense of both big toes was very poor, and co-ordination of movement in the lower limbs was severely impaired. Vibration sense was absent from the anterior superior iliac spines and distal points, and he was unable to stand without swaying, even with his eyes open and his feet apart.

From the 43rd to the 75th day he was given 300 $\mu\text{g.}$ of vitamin-B₁₂-peptide. This was then reduced to 200 $\mu\text{g.}/\text{day}$, and remained unchanged up to the time of writing (445th day).

After 10 weeks the sensation in the fingers had become normal. Tightness and numbness had disappeared completely from the abdomen and had lessened in the legs. Gait had improved, and he could stand with feet together and eyes closed for 10 seconds without overbalancing.

After 24 weeks numbness in the lower limbs had become confined to anterior surfaces of the thighs and lateral halves of the feet. The knee-jerks had returned and the vibration sense was present over the anterior superior iliac spines. Romberg's test showed very slight sway but no overbalancing after 30 seconds. He was walking normally and could also run about 15 yards. Co-ordination of both limbs was good, and postural sense had returned to the toes. After one year the ankle-jerks returned, and the numbness of the feet became noticeable only when he was tired. Vibration sense had also returned to both knees, but some tightness still persisted in the thighs. The right plantar response had also become flexor by this time.

At the time of writing the haemoglobin was 116%, P.C.V. 59%, and serum-vitamin-B₁₂ level 320 $\mu\text{g.}/\text{ml.}$ The blood urea was 40 $\text{mg.}/100 \text{ ml.}$

Case 67

A man aged 58 had, on admission to hospital, a Hb value of 39%, a P.C.V. value of 16%, and a serum-vitamin-B₁₂ level of 40 $\mu\text{g.}/\text{ml.}$ Numbness of the fingers and toes had been present for two months in addition to symp-

toms of anaemia. He was unable to feel the edges of the pages of a book or to fasten buttons. He also noticed that his right foot was stiff and weak, and when he had walked about 100 yards (90 metres) it tended to drop and became difficult to control. Flexion and extension of the toes of both feet were difficult and painful. Vibration sense was absent over the big toes, but no other abnormal physical sign was detectable.

From the 1st to the 202nd day he was given 40 $\mu\text{g.}$ of vitamin-B₁₂-peptide complex daily, and this dosage was then reduced to 20 $\mu\text{g.}/\text{day}$.

A good reticulocyte response occurred (maximum 20% on the 7th day) and the Hb level rose to 86% and the P.C.V. to 38.5% (53rd day). Numbness of the fingers and toes disappeared quickly, and after two months the vibration sense returned to the big toes, and the stiffness and weakness of the lower limbs disappeared. He remained symptom-free and returned to work as a clerk. On the 316th day his Hb was 90%, P.C.V. 41%, and serum-vitamin-B₁₂ level 55 $\mu\text{g.}/\text{ml.}$

Case 68

A woman aged 58 noticed increasing weakness and stiffness of the legs, with numbness of the hands and feet during 18 months. On examination she was severely ataxic, and could walk only if she held on to furniture. She was quite unable to stand without support—even with her eyes open. Knee- and ankle-jerks were increased, the left plantar response was extensor, and the right was "doubtful" extensor. The joint movements in the lower limbs were stiff and vibration sense was absent over the knees and below. Co-ordination of the leg movements was poor, and sense of position of the great toes was severely impaired. In addition to the numbness of the hands and fingers, she was unable to write, sew, or knit.

At the beginning of treatment the Hb was 75% and P.C.V. 34%. A fractional test-meal revealed a histamine-fast achlorhydria, and sternal marrow examination revealed partially megaloblastic erythropoiesis. The serum-vitamin-B₁₂ level was 50 $\mu\text{g.}/\text{ml.}$

Treatment was commenced with 300 $\mu\text{g.}$ of vitamin-B₁₂-peptide daily for eight days, when it was reduced to 200 $\mu\text{g.}/\text{day}$. This remained unchanged up to the time of writing (313th day).

A reticulocyte response was obtained (maximum 26% on the seventh day) and on the 33rd day the Hb was 89% and the P.C.V. 43%.

After two weeks the numbness receded to the distal phalanges of the fingers and to the distal halves of the feet. Hand movements became more controlled. The right plantar response became flexor after three weeks' treatment. By the end of the six weeks she was able to walk 50 yards (45 metres) without assistance, and after six months her gait was practically normal and she was able to stand without swaying when her feet were together and her eyes closed.

After 10 months her vibration sense had returned to the patellae and proximal halves of the tibiae, and residual numbness was confined to the tips of the fingers. The feet by this time felt normal. The left plantar response was still extensor, but joint-sense and co-ordination of movement of the lower limbs were excellent.

At the time of writing the Hb was 96%, P.C.V. 46%, and serum-vitamin-B₁₂ level 320 $\mu\text{g.}/\text{ml.}$

Case 71

A woman aged 55 gave a history that some 14 years previously she had noticed a weakness and numbness of the legs, accompanied by progressive ataxia. "Anahaemin" 2 ml. twice a week was given, and she recovered sufficiently to resume work as a daily help. Five years ago, however, she stopped having injections, and later began to deteriorate. Twice-weekly injections of anahaemin (2 ml.) were recommended 18 months ago, but no improvement occurred this

time. It was at this stage that she was first seen by one of us (F. S. M.). She could walk only a few steps, touching furniture as she went. She was unable to stand without support, even with her eyes open and her feet apart. Her lower limbs were cold and numb. There was considerable muscle-wasting in the thighs and legs; and marked degree of spasticity. Vibration sense was absent over both anterior superior iliac spines and below, knee- and ankle-jerks were exaggerated, and the plantar responses were extensor. The sense of position of both big toes was poor. Her upper limbs were not affected. When first seen her haemoglobin was 109%, P.C.V. 47%, and serum-vitamin-B₁₂ level 300 $\mu\text{g./ml.}$

After 300 $\mu\text{g.}$ of vitamin-B₁₂-peptide complex daily for nine months coldness of the feet had disappeared, but the remaining signs and symptoms were unchanged.

Case 72

A man aged 56 was admitted to hospital because of increasing stiffness and loss of power in the legs, culminating in his complete inability to walk even when supported. Numbness was present in the hands, and he was unable to write or to use a knife and fork. Symptoms had been present for a year. On examination severe sensory impairment was observed from the waist downwards and in the forearms, hands, and fingers. Co-ordination of leg movements was extremely poor and muscles were very weak. He was unable to alter his position in bed without help, and could not feel his feet touching the ground. Knee- and ankle-jerks were exaggerated, and there was an unsustained ankle clonus. The right plantar response was doubtful, but the left was flexor. Vibration sense was absent over the anterior superior iliac spines and below. The heel-shin test was grossly abnormal.

The haemoglobin was 90% and P.C.V. 41%, but sternal-marrow examination revealed a megaloblastic erythropoiesis, and there was a histamine-fast achlorhydria. The serum-vitamin-B₁₂ level was 45 $\mu\text{g./ml.}$

For the first 270 days he received 300 $\mu\text{g.}$ of vitamin-B₁₂-peptide daily, and thereafter 200 $\mu\text{g./day}$ up to the time of writing (330th day).

After one month he could feel his feet touching the floor and the stiffness of the legs had become less. The hands had also improved and he could hold a cup safely in one hand, whereas previously he had needed two hands. Greater co-ordination in leg movements (heel-shin test) was evident, and he could make a bizarre attempt at walking if well supported by two people. Even so, his legs often became entangled one behind the other. Slow improvement was maintained, and after three months he could use a knife and fork and could walk about 20 paces with generous support from one person. By six months he was managing to walk with the aid of a walking-stick in one hand and a "tripod" in the other, and during the last four weeks he was using two ordinary sticks to assist his balance in walking. At the time of writing he could rise from the sitting to standing position without aid, but was still unable to walk without the help of his sticks. The numbness in the lower limbs had been replaced by a "tingling" sensation, which was slight in the morning and became more pronounced as the day wore on. The stiffness in the legs had also diminished and he could control his muscles, whereas in the early stages of treatment they used to "grip him" and he could not relax them. His hands were virtually normal apart from some residual tingling in the tips of the fingers. He could shave and write a letter. The power of the lower limbs was good, and he could lift 30 lb. (13.6 kg.) in weight with either leg. His Hb level was 110%, P.C.V. 47%, and serum-vitamin-B₁₂ level 440 $\mu\text{g./ml.}$

Case 77

The patient, a woman aged 69, was of heavy build and had marked bilateral osteoarthritis of the knees. She had

noticed an increasing numbness and weakness of the legs and hands for about three years. There was also some stiffness in the legs and shoulders, and at the time of admission to hospital she was unable to stand unaided, even with her feet apart and her eyes open. Ataxia was marked, and she could move only by touching furniture. She was unable to recognize objects placed in her hands, and she tended to drop things. She was unable to write, to knit, or to sew. Knee- and ankle-jerks were absent, vibration sense was lost over the knees and below, but plantar responses were flexor. She was severely anaemic—the Hb was 30% and P.C.V. 15%. The serum-vitamin-B₁₂ level was 30 $\mu\text{g./ml.}$

Vitamin-B₁₂-peptide 300 $\mu\text{g.}$ was given daily from the first to the 109th day, and then 200 $\mu\text{g.}$ daily from the 110th day until the time of writing (236th day).

The anaemia responded well, and after a month the Hb value had risen to 82% and P.C.V. to 42%. After six months the ataxia had diminished and she was able to stand alone with feet together and eyes open, although she still needed to steady herself by touching furniture when walking around the house. The knee-jerks reappeared, and vibration sense returned to the right knee. Paraesthesiae in the hands had diminished, but the movements were still clumsy and she still dropped things, though less frequently than before. The shoulder stiffness was unchanged.

Case 79

A housewife aged 53 complained of stiffness of the knees and ankles and of unsteady gait during the previous 12 months. Her Hb level was 63%, P.C.V. 28%, and serum-vitamin-B₁₂ level 36 $\mu\text{g./ml.}$ The marrow was megaloblastic. On examination Rombergism was pronounced, and she tended to fall almost immediately when her feet were placed together and her eyes were closed. Power in the lower limbs was good, knee- and ankle-jerks were normal, and plantar responses were flexor; vibration sense, however, was absent from the knee downwards in the left leg, and was diminished over the same area in the right leg.

She was given 30 $\mu\text{g.}$ of vitamin-B₁₂-peptide complex daily for 15 days, and, although the Hb rose to 80% and the P.C.V. to 34% during that time, no improvement in the neurological condition was noted and the dosage was increased to 300 $\mu\text{g./day}$. Six weeks later the Rombergism had disappeared completely and the vibration sense had returned to the left tibia. The stiffness in the joints was present at night but absent during the day. After four months the vibration sense returned to the left patella and the joint stiffness completely disappeared. On the 137th day she was symptom-free and showed no residual signs of nerve damage. The Hb was 110%, P.C.V. 42%, and serum-vitamin-B₁₂ level 500 $\mu\text{g./ml.}$

Discussion

The average age of the 11 patients (seven women and four men) was 59 years. The lower limbs were affected in all of the cases, and the upper limbs in seven. The duration of the symptoms ranged from two months to 14 years, but in most cases they had been present from 4 to 18 months. The daily average maintenance dose ranged from 30 to 300 $\mu\text{g.}$

In seven patients (Cases 7, 34, 40, 55, 67, 68, and 79) the subjective and objective responses were excellent, and they improved to lead normal lives with little or no residual effects. In Case 47 the response was good. In Cases 72 and 77 the responses were fair, but an appreciable degree of disability still persisted. In Case 71, in which the condition had been present for 14 years before oral therapy was started, the response was poor.

No attempt has been made in the series to compare this form of therapy with any other, but one or two

observations are perhaps worthy of mention. Firstly, whereas with crystalline vitamin B₁₂ very little further improvement can be expected after six months' parenteral therapy (Ungley, 1949), in most of our cases a steady improvement was noticed over the first two years of treatment. Secondly, on parenteral therapy, according to Denny-Brown (1959), it is "unlikely that signs that have been present for months can be reversed." In the present series, however, although the symptoms in 8 of the 11 cases were of six months' duration or more, some improvement in the initial signs occurred in all except one case (No. 71).

Finally, much has been heard during the past few years about the significance of vitamin-B₁₂ levels in the serum on vitamin-B₁₂-peptide therapy. It has been widely assumed that because low serum-vitamin-B₁₂ levels were found to be the gloomy heralds of haematological and neurological disaster when patients were receiving intrinsic-factor preparations, a similar interpretation of low levels was also justifiable on vitamin-B₁₂-peptide therapy.

Our preparations, however, are not intrinsic-factor preparations, and the assumption, therefore, that low serum levels have the same significance on both forms of therapy is still unproved. We believe, in fact (Mooney and Heathcote, 1961b; Heathcote and Mooney, 1961), that serum-vitamin-B₁₂ levels have little practical value in vitamin-B₁₂-peptide therapy, and in this connexion it is noteworthy that one patient (Case

67) has achieved complete recovery not only from all signs and symptoms of anaemia but also from nerve degeneration, although his serum-vitamin-B₁₂ level has never exceeded 55 µg./ml.

Summary

Eleven cases of subacute combined degeneration of the spinal cord were treated orally with vitamin-B₁₂-peptide preparations for periods up to four years.

The average dose ranged from 30 to 300 µg. (equivalent) of vitamin B₁₂ a day.

In seven cases an excellent response was obtained and all patients are leading normal lives.

Three patients showed fairly good responses, and one—in whom the disease had been present for 14 years—showed almost no improvement.

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SCREENING OF 12 GLIOMATA AGAINST CHEMOTHERAPEUTIC AGENTS IN VITRO

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Ambrose and his colleagues (1962) devised a method whereby the sensitivity of human tumour cells obtained at biopsy or operation, and grown in tissue culture, could be tested quantitatively against chemotherapeutic substances. As these are primary cultures, and have presumably changed little compared with cells *in vivo*, it was felt that information obtained in this way might be of value to the clinician in his choice of drugs for the treatment of individual cases.

Using the methods described in detail below, we have attempted to cultivate many types of tumours, and whereas we have had scant success with most carcinomata and sarcomata, our experience with gliomata, comprising astrocytomata, ependymomata, and medulloblastomata, has been almost invariably successful. The results obtained in the testing of 12 gliomata and a culture of HeLa cells are reported here.

Methods and Materials

The specimens examined were all obtained from Atkinson Morley's Hospital or the Hospital for Sick Children at Great Ormond Street. At operation the tumours are placed in either a dry sterile screw-capped bottle or into Parker's TC medium 199 and packed in ice in a vacuum flask. They are brought to the Chester Beatty Research Institute and arrive within the hour. Immediately on arrival the specimens are chopped up manually with fine scissors and, being very soft, fragment easily. The "mush" so obtained is transferred to a 0.06% solution of commercial trypsin ("difco") in Parker's TC medium 199 and agitated in a magnetic

stirrer for 30 minutes at 37° C. Thereafter the coarser particles in the suspension are allowed to settle and the supernatant fluid is pipetted off and centrifuged for five minutes at 1,500 r.p.m. The supernatant from this procedure, which contains the trypsin, is removed and the centrifuged cells are resuspended in the final medium, which consists of 20% of the patient's serum + 80% Evans N.C.T.C. medium 109, to which folic acid (0.4 mg./litre) and insulin (438 mg./litre) have been added. Repeated washings of the cells were found to give less satisfactory results, and it is felt that the trypsin inhibitors in the serum incorporated in the medium are sufficient to neutralize the traces of trypsin remaining after centrifugation.

The suspension thus achieved is dispensed in 0.3-ml. quantities into Ambrose tubes (Ambrose *et al.*, 1962) and incubated at 37° C. in a gas phase of 5% CO₂ in air. A good monolayer of cells on the base of the tubes has usually formed in 48 hours and the medium is then changed, giving a clear fluid through which observations with an inverted microscope can be made. The monolayer is almost always dense enough to allow counting to be performed with the Chalkley eyepiece (Curtis, 1960). The drugs selected for administration are then added according to the method already described (Ambrose *et al.*, 1962), the same volume—0.08 ml.—of Hanks's saline being added to the control tubes.

The drugs chosen for this series were: melphalan (*p*-di-(2-chloroethyl)amino-L-phenylalanine), thiotepe (triethylene thiophosphoramide), chlorambucil (*p*-di-(2-chloroethyl)aminophenylbutyric acid), mannitol busul-