age nor sex appeared to be a common factor accounting for the varying responses in individuals, but, in general, initial low levels of penicillin were followed by subsequent low ones and vice versa.

In one ward patient and in three ambulant patients a total of 38 penicillin estimations were made at intervals throughout 24-hour periods after injections of 300,000 units of procaine penicillin. In these patients, although the highest levels tended to appear in samples taken in the five hours following each injection, the high peak levels which occur shortly after the injection of most preparations of penicillin in oil and beeswax were not seen. The highest level reached was 1.44 units, and on the whole the amount of penicillin in the blood remained fairly constant throughout the 24 hours.

Although the series is a small one, the clinical response to this treatment has seemed to be at least equal to that seen in patients treated with injections of 250,000 units of penicillin in saline twice daily, and the clinical impression is that a number of lesions which probably would have gone on to suppuration with twice-daily injections of penicillin in saline have been aborted. It has proved possible, with one daily out-patient attendance and rest at home, to manage some patients who would otherwise have required admission.

Discussion

In the majority of 49 patients it has been possible with procaine penicillin to maintain detectable amounts of penicillin in the blood throughout treatment. There has been a certain amount of individual variation in the levels maintained. The variation noted has not been so great as that observed by Boger *et al.* (1948), and agrees more closely with that found by Herrell *et al.* (1947) and by Hewitt *et al.* (1948). Whether these variations are due to the use of the compound in different classes of patients or to variations in different batches of procaine penicillin is uncertain.

In the four cases followed more closely the release of penicillin was a gradual process, and, as pointed out by Hewitt *et al.* (1948), the high peak levels usually obtained after injections of most preparations of penicillin in oil and beeswax did not occur.

In the circumstances described here it appeared that, if it is desirable to maintain a low but constant amount of penicillin in the blood throughout treatment, this might be accomplished in the majority of ambulant patients with more certainty by means of procaine penicillin than by the use of penicillin in oil and beeswax, and with an absence of local reactions. Whether the failure of procaine penicillin to produce intermittent high blood concentrations of penicillin will constitute a drawback to its use as a therapeutic agent remains to be seen.

The number of cases treated is too small to draw any conclusions from the clinical results, but if a more extensive trial proved them to be satisfactory the use of a method of treatment with penicillin necessitating only one daily injection would allow the patient considerably more rest, and would have considerable advantages in general practice and in hospital out-patient departments. If procaine penicillin were also shown to be satisfactory for the treatment of in-patients a considerable amount of time in wards would be saved by the substitution of single daily injections for spaced three-, four-, or five-hourly injections of penicillin in saline.

As each dose of 300,000 units of procaine penicillin contains 124.5 mg. of procaine base, it is possible that those sensitive to procaine may show reactions from its use. No such reactions have yet been reported, and it is likely that, owing to the very slow rate of absorption into the circulation, toxic blood concentrations will not be reached.

Summary

Estimations of penicillin in the blood following the use of procaine penicillin G in oil in 45 ambulant patients and 4 ward patients are recorded.

In the majority of the patients penicillin could be detected in the blood between eighteen and twenty-six hours after daily injections of 300,000 units of procaine penicillin G in oil.

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CRYSTALLINE ANTI-PERNICIOUS- ANAEMIA FACTOR IN TREATMENT OF TWO CASES OF TROPICAL MACROCYTIC ANAEMIA

BY

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Recently, crystalline material found to be effective in the treatment of pernicious anaemia has been isolated from liver independently in two laboratories. In America, Rickes, Brink, Koniuszy, Wood, and Folkers (1948) isolated a red crystalline material which they named vitamin B₁₂, and which was shown by West (1948) to be clinically active in microgram quantities. A red crystalline substance which has been shown by Ungley (1948a) to possess in pernicious anaemia similar clinical activity to that described by the American workers has also been isolated in England (Lester Smith, 1948; Lester Smith and Parker, 1948).

Spies, Stone, and Aramburu (1948) treated five patients—two with pernicious anaemia, two with nutritional macrocytic anaemia, and one with non-tropical sprue—with doses of 6 to 15 µg. of vitamin B₁₂, and in all instances haemopoietic responses were observed. Spies, Lopez, Milanes, Toca, and Culver (1948) successfully treated two cases of tropical sprue with 8 µg. of vitamin B₁₂. Ungley (1948a, 1948b) found that in pernicious anaemia single doses of 10 µg. of Lester Smith's red crystalline material induced maximal reticulocyte responses and a rapid increase in red blood cells. Preliminary observations suggested that the material was as effective as liver extract in subacute combined degeneration. However, he recorded that a patient with megaloblastic anaemia of pregnancy did not respond to 65 µg. although she subsequently responded to 2.5 mg. of folic acid daily (Ungley, 1948a).

This paper reports the effect of treatment of two cases of tropical macrocytic anaemia, as occurring in Bombay, with Lester Smith's crystalline anti-pernicious-anaemia factor. The patients were admitted to the Singhanee Hindu Hospital. Detailed medical and dietary histories were obtained and thorough medical examinations carried out. The investigational procedure employed was that described

by Bhende and Patel (1946). The diet provided consisted of milk, bananas, vegetables, bread, rice, and dal. Earlier work (Patel, 1946) has shown that this diet produces no haemopoietic effect.

Haematological examinations of red cells, white cells, corpuscular volume, and haemoglobin were carried out every other day; reticulocyte counts were made daily. In each case bone marrow was obtained by sternal aspiration before treatment and on the day following the reticulocyte crisis.

Both patients received single injections of a solution containing 80 µg. of Lester Smith's crystalline factor and in both instances good responses were observed. There were pronounced reticulocyte responses. The red blood cells and haemoglobin increased and the bone marrow changed from megaloblastic to normoblastic (see Tables I and II).

TABLE I.—Haemopoietic Response of Tropical Macrocytic Anaemia to Injection of 80 µg. of Crystalline Anti-pernicious-anaemia Factor.

Case No.	R.B.C. (millions per c.mm.)		W.B.C. (per c.mm.)		Haemoglobin (g. per 100 ml.)		Reticulocytes	
	Initial	After 14 Days	Initial	After 14 Days	Initial	After 14 Days	Day of Peak	%
1	1.68	2.56	4,100	6,500	8.65	11.24	6	11.5
2	1.76	2.56	2,500	4,600	6.92	9.51	5	36.8

TABLE II.—Response of Bone Marrow to Treatment with Crystalline Anti-pernicious-anaemia Factor.

	Case 1		Case 2	
	Before Treatment	After 7 Days	Before Treatment	After 6 Days
Proerythroblast ..	1.0	0.4	1.0	0.4
Megaloblast { Type 1	1.0	0.0	13.0	0.0
Type 2	4.2	0.0	12.2	0.0
Type 3	6.4	1.2	2.4	3.4
Normoblast { Type 1	0.0	0.0	0.2	1.0
Type 2	2.6	2.8	0.0	8.0
Type 3	5.0	28.8	13.2	44.4

Case 1

A male shop assistant aged 42, of poor economic status, was admitted because of general weakness of twelve months' standing which had increased during the past seven months and which had prevented him from working for three months. For twelve months he had passed two to three stools daily and there had been much flatulence and pain in the left side of the abdomen. Stomatitis had been present throughout and was more severe during the seven months before admission. He had suffered from giddiness and palpitation for three months; there was no paraesthesia. The family history was negative and there had been no previous illness of any significance. He was a vegetarian, and had been on a self-imposed starchy diet on account of "indigestion." He stated that he had been treated with some liver preparation two months before admission.

When admitted he was emaciated and pale and his skin was sallow. There was nothing abnormal in the respiratory or nervous system. The tongue was pale and smooth and there was no abnormal sign in the abdomen. The urine and stool showed no abnormality, and the blood Wassermann reaction was negative. Gastric analysis (oatmeal) revealed the presence of free hydrochloric acid. The blood picture before treatment was as follows: red cells, 1,680,000 per c.mm.; haemoglobin, 8.65 g. per 100 ml.; white cells, 4,100 per c.mm.; M.C.V., 140 c.µ, M.C.H.C., 36%. The differential leucocyte count showed: polymorphonuclear leucocytes 55%, eosinophils 4%, mononuclears 2%, and lymphocytes 39%. The indirect van den Bergh reaction was negative, and the icterus index 7 units. After the injection of 80 µg. of crystalline anti-pernicious-anaemia factor a reticulocyte maximum of 11.5% occurred on the sixth day, and after 14 days the red blood cells had increased to 2,560,000 per c.mm., the haemoglobin to 11.24 g. per 100 ml., the white cells to 6,500 per c.mm.; the M.C.V. was 125 c.µ, and the M.C.H.C. 34.5%.

The marrow was examined before treatment and seven days after the injection; Israël's (1939) terminology was used. The results given in Table II show that the treatment resulted in a change from megaloblastic to normoblastic.

The patient's appetite began to return on the third day after the injection, and by the sixth day had increased tremendously. His diet consisted now of bread, bananas, and milk. His weight increased from 91 lb. (42.27 kg.) on admission to 97 lb. (44.99 kg.) after one week. In spite of this improvement in general condition and blood picture he developed an attack of stomatitis ten days after the injection which prevented him from eating bread and bananas, and hence he lost 3 lb. (1.36 kg.) during the second week. However, his general appearance had improved considerably.

Case 2

A schoolboy aged 16 was admitted suffering from general weakness, loss of appetite, and stomatitis, which had persisted for two months; there had been low fever for one month. He had had an attack of dysentery at the age of 10, and since then he had had intermittent pain in the abdomen and diarrhoea. Six months before admission he had had a recurrence of dysentery, for which he was given a special diet and received symptomatic treatment. There was another attack of diarrhoea three months before admission, and the symptoms for which he was admitted dated from about that time. He had received no liver therapy. For the last two months he had suffered from flatulence and passed one or two loose stools in the morning. On account of this he had restricted his diet to rice and curdled milk. There was nothing of importance in his family history.

He was pale and sallow. On examination no abnormality was detected in his respiratory, cardiovascular, nervous, or alimentary system. Urine was normal; the stool examination showed Charcot-Leyden crystals, with vegetative forms of *Trichomonas hominis* but no other protozoa or ova. The Wassermann reaction was negative; gastric analysis (oatmeal) revealed the presence of free acid. The blood picture before treatment was as follows: red cells, 1,760,000 per c.mm.; M.C.V., 105.1 c.µ; M.C.H.C., 37.4%; haemoglobin, 6.92 g. per 100 ml.; white cells, 2,500 per c.mm. The differential leucocyte count was: polymorphonuclear leucocytes 58%, eosinophils 4%, basophils nil, large mononuclear leucocytes nil, lymphocytes 38%. The van den Bergh reaction (direct and indirect) was negative; the icterus index was 5 units. He was given a single injection of a solution containing 80 µg. of crystalline anti-pernicious-anaemia factor. A reticulocyte maximum of 36.8% occurred on the fifth day. Appetite began to return on the third day, and on the fourth day he felt very hungry. He was given a diet of bananas, bread, and milk, and in 14 days he increased in weight by 8 lb. (3.63 kg.).

The blood picture on the fourteenth day after the beginning of treatment was: red cells, 2,560,000 per c.mm.; M.C.V., 101.5 c.µ; M.C.H.C., 36.5%; haemoglobin, 9.51 g. per 100 ml.; white cells, 4,600 per c.mm. The marrow, which was megaloblastic before treatment, had become normoblastic six days after the injection (see Table II).

Summary

Two cases of tropical macrocytic anaemia showed good responses to treatment with 80 µg. of Lester Smith's crystalline anti-pernicious-anaemia factor.

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