SIDE-EFFECTS OF A PREPARATION OF VITAMIN B₁₂

BY

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During the course of an investigation involving large numbers of intramuscular injections of a proprietary preparation of vitamin B₁₂ (Bedford, 1951a) side-effects were noticed which warranted further inquiry. This preparation was a vitamin-B₁₂ "concentrate" derived from *Streptomyces griseus* fermentation liquors, and, being both effective and inexpensive, was assuming increasing prominence in the treatment of pernicious anaemia. It was of interest to determine whether the vitamin B₁₂ itself or a contaminant was responsible for these side-effects, and to investigate some factors which may have predisposed to their occurrence.

Method

Three preparations were available for trial: (1) "A" was the proprietary vitamin-B₁₂ preparation referred to above, containing 20 µg. per ml. It is described as a concentrate, being in fact a solution of vitamin-B₁₂ factors derived from streptomyces fermentation liquors by a combination of customary extraction procedures, and is no longer in general issue (J. T. Marsh, 1951, personal communication). (2) "B" was a proprietary preparation of vitamin B₁₂ derived from liver and contained 10 µg. of B₁₂ per ml. (3) "C" was a solution of vitamin B₁₂ containing 20 µg. per ml., derived, like A, from streptomyces culture liquors, but "purified by crystallization and therefore likely to contain a smaller proportion of any impurity" (J. T. Marsh).

At the time of this investigation the proprietary preparation which has replaced A in general issue was not available for trial. It is, like C, a streptomyces derivative purified by crystallization, but is a solution of vitamin B₁₂ (not B₁₂), and its impurities are stated not to exceed 1 part in a million millions (J. T. Marsh, 1951; H. M. Walker, 1951: personal communications).

One hundred people (27 women and 73 men) between the ages of 21 and 90 were injected intradermally with 0.1 ml. of A, 0.2 ml. of B, and 0.1 ml. of C—that is, equal doses of vitamin B₁₂—on the flexor surface of the forearm, and the results recorded at 15 minutes, 30 minutes, and 24 hours. Positive reactors were arbitrarily assessed as those with persistent wheals 5 mm. and more in diameter.

Results

The results are set out in Table I. There were 14 positive reactions to A, none to B, and two to C (both of these being also positive to A).

TABLE I.—Positive Intradermal Tests

		0.1 ml. A	0.2 ml. B	0.1 ml. C
Men	73	9	0	1
Women	27	5	0	1
Total	100	14	0	2

Of the 100 people under test, 52 had received one or more antibiotics within the past five years (49 penicillin, 7 streptomycin, 4 "aureomycin," and 2 chloramphenicol), while 48 denied previous contact with mould products. Table II illustrates the relationship between skin-test positive reactions and previous treatment with antibiotics. Of those

TABLE II.—Relation Between Positive Intradermal Reactions and Previous Antibiotics

	Previous Antibiotics	A-positive	C-positive	No Previous Antibiotics	A-positive	C-positive
Men	43	6	1	30	3	0
Women	9	4	1	18	1	0
Total	52	10 (19%)	2	48	4 (8%)	0

who had previously received antibiotics, 19% were skin-test-positive to A, whereas only 8% of those who had not previously received antibiotics were A-positive.

As the relationship between positive intradermal reactions and general sensitivity phenomena is not absolute, it was decided to estimate directly the proportion of people who might be expected to exhibit side-effects to A, B, and C when these were administered, as is usual, by deep intramuscular injection. Two groups of people were selected, group 1 being the 14 skin-test A-positive reactors, and group 2 being 14 skin-test A-negative reactors. They were injected with 2 ml. of A, 4 ml. of B, and 2 ml. of C—that is, 40 µg. of vitamin B₁₂ in each case—as follows:

Experiment 1: 2 ml. of A to group 1, and 2 ml. of A to group 2; followed two days later by *Experiment 2:* 4 ml. of B to group 1, and 4 ml. of B to group 2; followed two weeks later by *Experiment 3:* 2 ml. of C to group 1, and 2 ml. of C to group 2; followed after a further two days by *Experiment 4:* 2 ml. of A to group 1, and 2 ml. of A to group 2.

The results are set out in Table III. As experiments 2 and 3 yielded no reactions they are omitted from the table; the results of experiments 1 and 4 were identical and so are not set down separately. There were no side-effects to B

TABLE III.—Side-effects to A in Positive and Negative Skin Reactors

Side-effect of 2 ml. A Intramuscularly	A-positive	A-negative
Moderate	2	0
Mild	2	2
Nil	10	12
Totals	14	14

or C, but six cases reacted adversely to A. There were two moderately severe reactions (both in group 1), consisting of considerable pain and tenderness at the site of injection, with fever, shivering, and malaise beginning six hours after the injection and lasting 24 hours. Four mild reactions occurred (two in group 1, and two in group 2), consisting of pain and tenderness at the injection site lasting 24 hours.

Table IV illustrates the relationship between previous antibiotic treatment and the occurrence of side-reactions, and is based on experiments 1 and 4 above—that is, on 28

TABLE IV.—Relation Between Side-effects to A and Previous Antibiotics

	Previous Antibiotics		No Previous Antibiotics	
	No.	Side-effects	No.	Side-effects
Men	13	3	6	1
Women	6	2	3	0
Total	19	5 (26%)	9	1 (11%)

people. Of those who had previously received antibiotics 26% developed side-effects to intramuscular injection of A, whereas only 11% of those who had not previously received antibiotics reacted adversely.

Conclusions.—Although the numbers involved in these experiments are too few to be statistically evaluated, the following inferences may be drawn: (1) It is clear that the factor responsible for both the sensitivity reactions and the

side-effects was not vitamin B₁₂ but an impurity present in A, absent in B, and present in traces in C. This substance was therefore probably a carry-over impurity from the streptomycetes fermentation liquor (Tables I and III). (2) People who had previously received penicillin or other antibiotics were more than twice as likely to be skin-sensitive and to develop side-effects to the "mould impurities" as those who had not been so treated (Tables II and IV).

Discussion

There is increasing evidence that unusual responses to antibiotic therapy are nearly always the result of acquired sensitivity (*Lancet*, 1951), and that non-specific hypersensitivity to fungi or mould-products may be responsible for a significant proportion of these unusual responses. The conclusions above imply an antigenic relationship between antibiotics and carry-over impurities in certain vitamin-B₁₂ preparations derived from streptomycetes: it is a reasonable corollary that the converse relationship is valid.

In themselves, the side-effects and skin reactions described above, being trivial, would be scarcely worthy of mention were it not that they indicate yet another way in which idiosyncrasy to antibiotics may be acquired. Deaths directly due to this cause are very rare, but it may prove a serious handicap in the management of infections, since people who are idiosyncratic to one antibiotic may be sensitive to all the others (Bedford, 1951b) and reactions may be so severe that antibiotic therapy becomes impossible.

This investigation does not exclude the possibility that preparations of vitamin B₁₂ derived from liver may also contain impurities capable of producing sensitivity phenomena (nor, indeed, that the pure vitamin itself may be capable of doing so); but there is no evidence that sensitivity to liver derivatives implies idiosyncrasy to antibiotics.

Cogent reasons for preferring any preparation of vitamin B₁₂ to liver extracts were low price, greater purity, relative freedom from side-effects, and the trivial nature of these when they did occur. As, however, there are now available highly purified vitamin-B₁₂ preparations from both liver and streptomycetes, against which no evidence of liability to promote idiosyncrasy to antibiotics has been adduced, the use of the cruder mould-derivatives is unjustified.

Summary

Of 100 people 14 were skin-test-positive to a vitamin-B₁₂ concentrate derived from *Streptomyces griseus*, and six suffered side-effects after intramuscular injection.

Two were skin-test-positive to a purer preparation of vitamin B₁₂ derived by crystallization from streptomycetes cultures, and none developed side-reactions to intramuscular injection.

None reacted adversely to a preparation of vitamin B₁₂ derived from liver.

Impurities carried over from the mould fermentation liquor are regarded as responsible for the sensitivity phenomena.

Both cutaneous reactions and side-effects were more than twice as common in people who had previously been treated with antibiotics.

The possibility of inducing idiosyncrasy to antibiotics is advanced against the use of impure preparations of vitamin B₁₂ derived from mould cultures.

It is with great pleasure that I express my gratitude to Mr. J. T. Marsh and Dr. H. M. Walker, of the Medical Department of Glaxo Laboratories Ltd., for supplies of vitamin-B₁₂ preparations and for information concerning them; and to the patients and staff of the Cowley Road Hospital for their co-operation in this investigation.

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"EPIDEMIC" ABDOMINAL COLIC ASSOCIATED WITH STEATORRHOEA

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Many specimens of faeces from cases of gastro-enteritis and diarrhoea suspected of being infective in origin are sent to the laboratory for examination. Often a known bacterial or protozoal pathogen is found, or food-poisoning organisms are incriminated; but a number of sporadic cases and a few epidemics remain for which no satisfactory explanation is found. We have observed the occurrence of pale stools and steatorrhea among some of these unexplained cases, and an outbreak of gastro-enteritis of this kind is described here because it suggests the occurrence of a syndrome of infective intestinal disorder which is not generally recognized.

The Outbreak

In December, 1950, a number of cases of diarrhoea occurred in a day nursery accommodating 50 children under the age of 5. The course of events is summarized in the Table. After the tenth day no further cases of

Day of Outbreak	Case No.	Sex	Onset of Symptoms
1	1	M	Diarrhoea during night
	2	F	" and vomiting in morning
2	3	M	" " " " "
	4	F	" " at midday " " "
	5	M	Severe stomach-ache in afternoon
3	6 to 16		Diarrhoea beginning at various times during the day. Case 13 had also severe vomiting
4	17	F	Diarrhoea
	18	M	Diarrhoea
5	19 to 22		Four of the staff complained of abdominal pain and flatulence with a sensation of "churning up" in the intestines.
6	23	F	Diarrhoea
	24	M	Diarrhoea
	25 to 26		Two more of the staff felt ill
7	27	M	Diarrhoea and vomiting
	28	F	" " "
	29	M	Diarrhoea " " "
8	30	M	" " "
9	31	M	Vomiting
			Case 6 suffered from vomiting. She had been well for the three previous days
			Case 8 had diarrhoea again. He had been well for the five previous days
10			Case 3, who had been ill for four days and better for four days, developed a second bout of diarrhoea

diarrhoea or vomiting occurred. It will be seen that fresh cases developed over a period of nine days. In all, 25 children aged from 1 to 4 years and six staff were affected—just under half the number of persons at risk. In addition four household contacts of three children (Cases 2, 3, and 6) were affected. These secondary cases developed from two to four days after the onset in the child concerned.

Clinical Picture.—The clinical picture was characteristic. The affected children showed a striking pallor with the onset of symptoms, and pain in the abdomen or vomiting (five cases) was the first complaint. The urgency of the call to stool and the apparent straining were severe, out of proportion to the degree of the diarrhoea; and a few children who cried for their pots failed to pass any faeces. The adults who were affected suffered from abdominal pain, flatulence, and nausea, and felt ill; but no adult had frank diarrhoea.

No case was ill for longer than four days, but three cases relapsed and had a second bout of diarrhoea or vomiting a week after the onset of their first attack. Six of the children had slightly raised temperatures. One child had jaundice a fortnight after his diarrhoea.