CHAPTER III

RESULTS

1. Reproducibility.

A serum pool was simultaneously analysed 20 times by the radioisotope dilution and coated charcoal technique and the result is shown in Table 2.

Table 2.

The reproducibility of vitamin B_{12} estimated 20 times in a serum pool.

No.	Vitamin B_{12} in pooled serum (pg/ml).	No.	Vitamin B_{12} in pooled serum (pg/ml).
1	105.78	11	109.39
2	99.73	12	110.33
3	104.22	13	106.72
4	108.60	14	104.85
5	107.97	15	109.55
6	111.91	16	104.07
7	102.05	17	101.90
8	104.22	18	107.66
9	105.00	19	107.97
10	107.82	20	105.16

* pg = picogram

A mean value of serum vitamin B_{12} was found to be 106.0 pg/ml with a range of 99.73 to 111.91 pg/ml and a standard deviation of 3 pg/ml.

2. Recovery of added vitamin B_{12}

Sera with known vitamin B_{12} concentrations were mixed with the aqueous vitamin B_{12} standard and the estimated values of the mixed concentration were compared with theoretical concentrations. The results of the recovery experiments are shown in Table 3 and Figure 2. There was a very high percentage of recovery in the present experiments, i,e, 93 to 104 % for the vitamin B_{12} concentrations ranges from 575 to 3,000 pg/ml. These recoveries may be considered satisfactory, especially if one took account of the minuteness of the quantities involved.

3. Vitamin B_{12} concentration in samples.

3.1 Vitamin B_{12} concentration in fish sauce.

The results of vitamin B_{12} concentration in 108 samples of fish sauce obtained from markets in Bangkok are shown in Table 4. There is a wide variation of vitamin B_{12} content in the fish sauce ranging from 0.30 µg% to 5.92 µg % with a mean of 1.91 µg %. The frequency distribution of these values is shown in Figure 3.

Table 3.

Recovery experiments after adding the known amount of vitamin B_{12}

	Theoretical value (pg/ml)	Duplicated determined value (pg/ml)	Percentage recovery
0.5 ml of serum..	80.0	80.0	100.0
...+0.1 ml of std.B_{12}	575.2	544.7, 534.1	94.7, 92.8
...+0.2 ml of std.B_{12}	1070.4	1041.9, 1033.9	97.4, 96.6
...+0.3 ml of std.B_{12}	1545.6	1487.3, 1565.8	96.2, 101.3
...+0.4 ml of std.B_{12}	2060.8	2048.5, 2143.3	99.4, 104.0
...+0.5 ml of std.B_{12}	2556.0	2556.3, 2438.8	100.0, 95.4
...+0.6 ml of std.B_{12}	3031.2	2998.3, 3004.3	98.9, 99.1

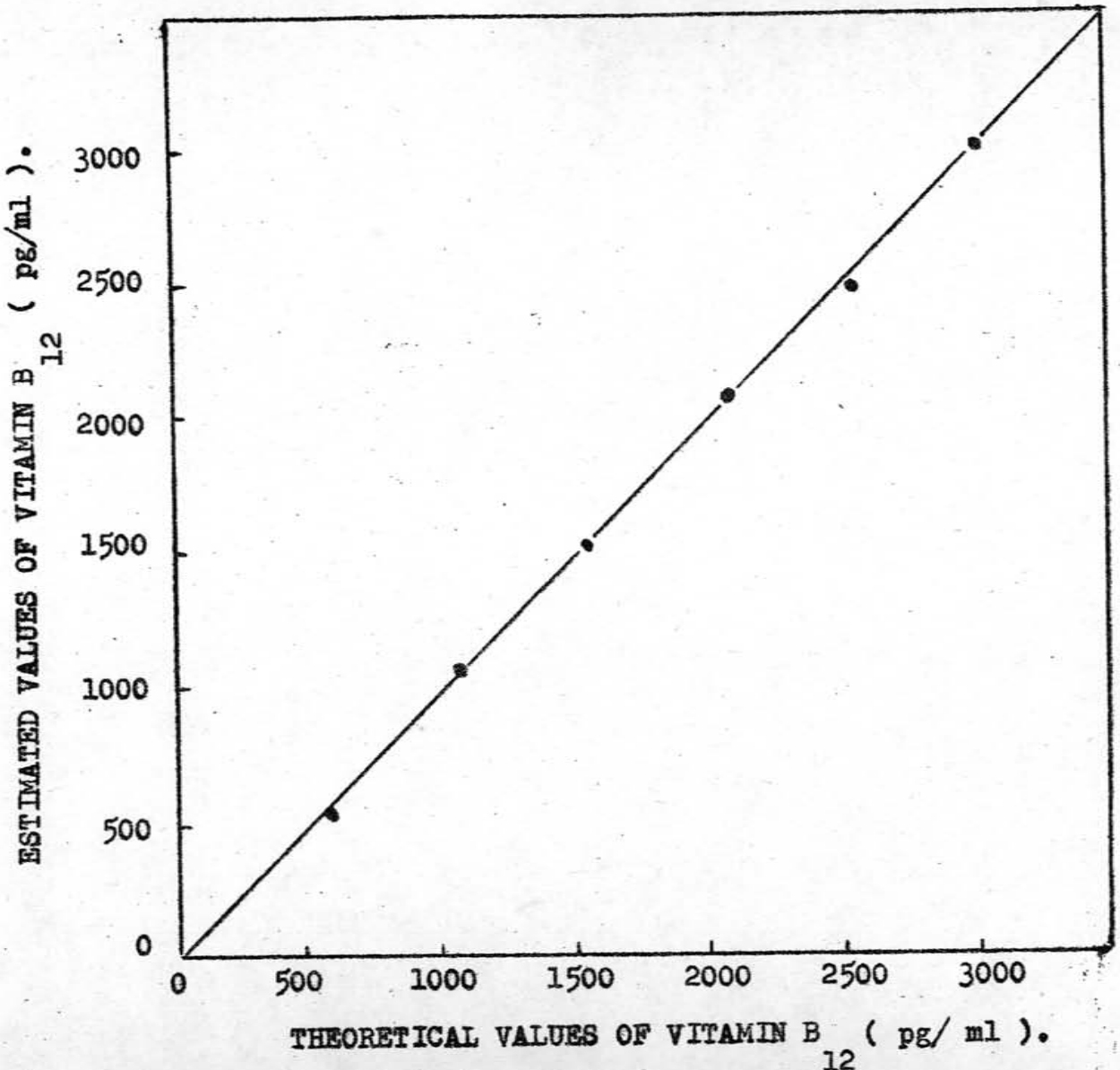

Fig. 2. Relationship between theoretical values and estimated values of vitamin B_{12}.

Table 4.

Vitamin B_{12} concentrations in 108 samples of fish sauce.

Sample No	Vitamin B_{12} (µg %)	Sample No	Vitamin B_{12} (µg %)	Sample No	Vitamin B_{12} (µg %)
1	1.72	19	1.04	37	2.18
2	5.01	20	1.84	38	0.84
3	1.50	21	4.18	39	2.27
4	2.79	22	0.96	40	5.79
5	2.63	23	3.10	41	1.04
6	1.19	24	1.12	42	1.11
7	0.73	25	1.23	43	0.76
8	5.82	26	1.60	44	3.17
9	4.94	27	2.03	45	1.57
10	3.79	28	2.44	46	0.95
11	0.99	29	3.03	47	1.18
12	3.37	30	0.74	48	1.64
13	5.04	31	0.41	49	1.53
14	0.92	32	0.69	50	2.98
15	2.02	33	1.57	51	1.97
16	2.10	34	2.60	52	1.49
17	4.06	35	1.67	53	1.18
18	0.31	36	2.47	54	4.22

Table 4 (Continued)

Vitamin B_{12} concentrations in 108 samples of fish sauce.

Sample No.	Vitamin B_{12} (µg %)	Sample No.	Vitamin B_{12} (µg %)	Sample No.	Vitamin B_{12} (µg %)
55	1.07	73	1.19	91	2.01
56	3.12	74	0.50	92	2.57
57	0.84	75	1.62	93	2.31
58	2.64	76	0.45	94	0.99
59	0.81	77	0.92	95	1.57
60	1.60	78	1.83	96	1.95
61	5.92	79	2.84	97	1.42
62	1.76	80	0.30	98	2.10
63	1.33	81	2.72	99	0.97
64	1.85	82	2.39	100	0.78
65	1.35	83	2.07	101	0.78
66	1.37	84	0.46	102	1.85
67	1.46	85	1.48	103	1.13
68	1.56	86	2.14	104	3.05
69	3.98	87	0.68	105	1.06
70	3.18	88	1.35	106	1.19
71	1.62	89	1.67	107	0.44
72	1.25	90	0.43	108	0.85

$N = 108$, $\bar{X} \pm SD = 1.91 \pm 1.24$, Range $= 0.30\text{-}5.92$

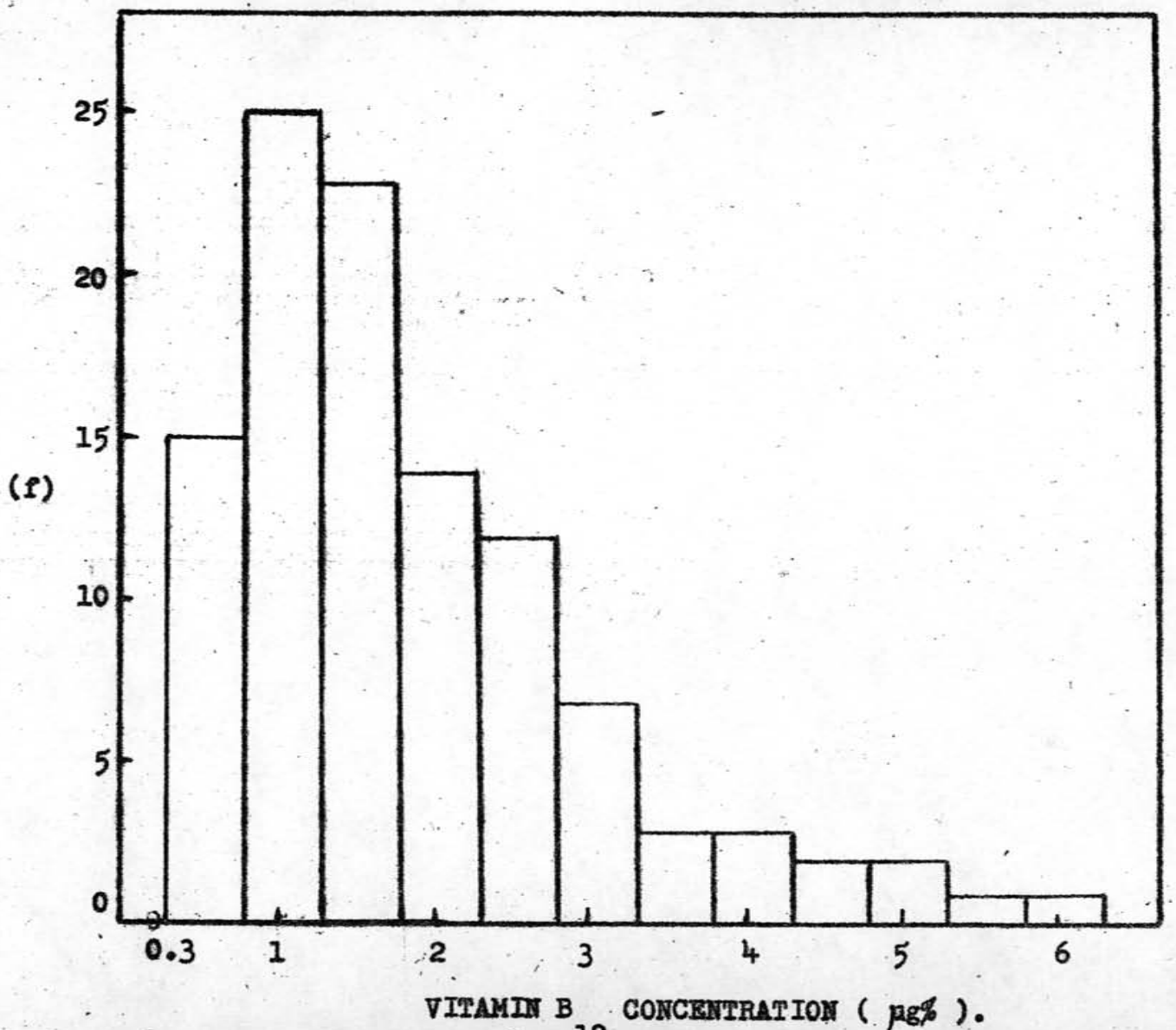

Fig. 3. Frequency distribution of vitamin B_{12} concentration in 108 samples of fish sauce.

3.2 Vitamin B_{12} concentration in soya-bean sauce.

Vitamin B_{12} concentrations were determined in 48 samples of soya-bean sauce obtained from markets in Bangkok. Thirteeen samples contained no vitamin B_{12} while 35 samples had a range of 0.01 µg % to 0.53 µg % with a mean of 0.13 µg %. The frequency distribution of these values are shown in figure 4 and Table 5. There is no relationship between the vitamin B_{12} concentrations in the soya-bean sauce samples and their prices as shown in Fig.5

Table 5

Vitamin B_{12} concentrations in 35 samples of soya-bean sauce.

Price (Baht/bottle)	No.of sample examined.	Vitamin B_{12} concentration(µg %)	
		Mean	Range
1.50-2.00	6	0.267	0.014-0.528
2.50-3.00	10	0.146	0.027-0.216
3.50-4.50	4	0.045	0.011-0.120
5.00-6.00	7	0.150	0.010-0.427
8.00-25.00	4	0.050	0.009-0.080
Total	35	0.137	0.010-0.528

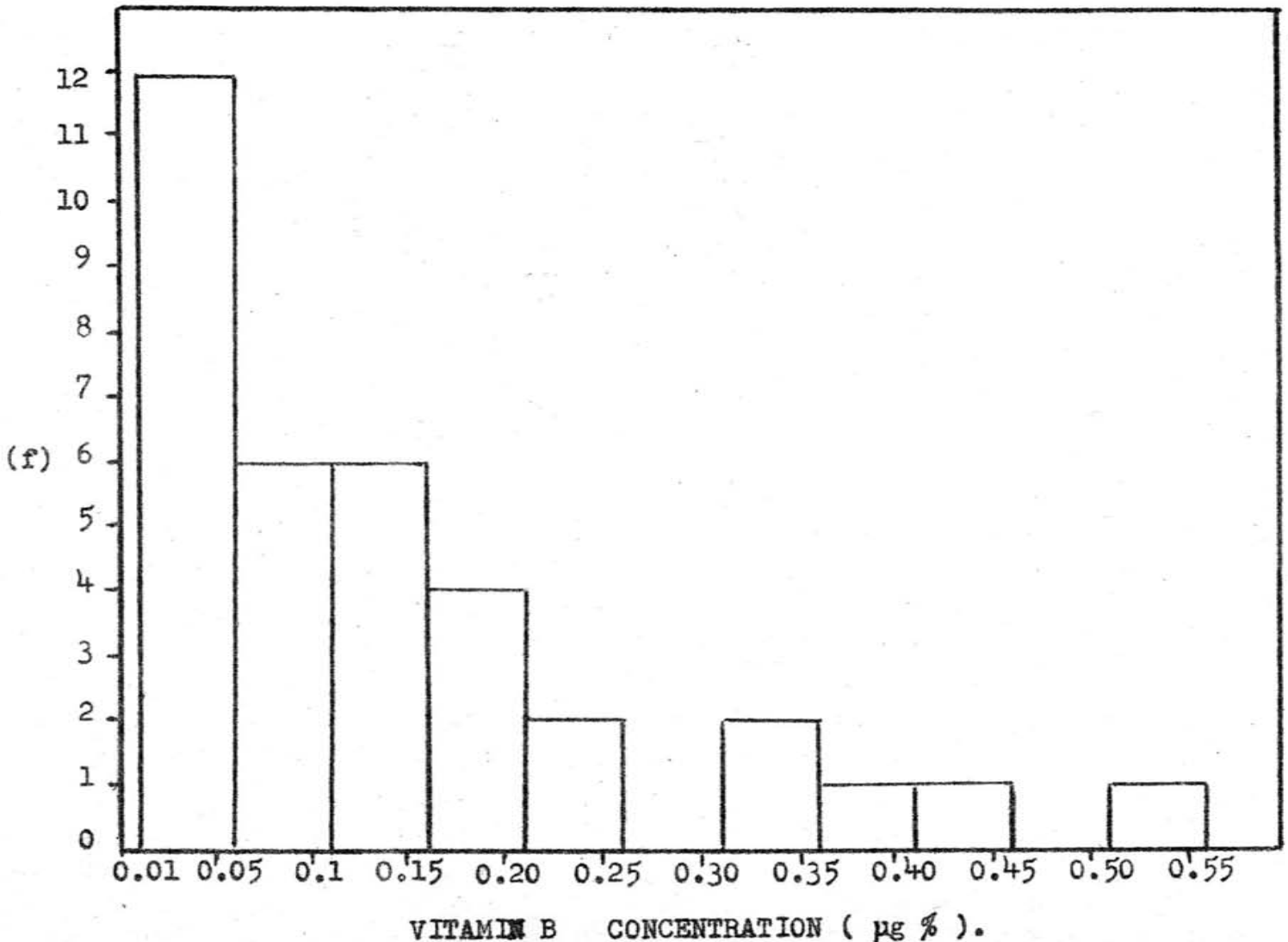

Fig. 4. Frequency distribution of vitamin B_{12} concentration in 35 samples of soya-bean sauce.

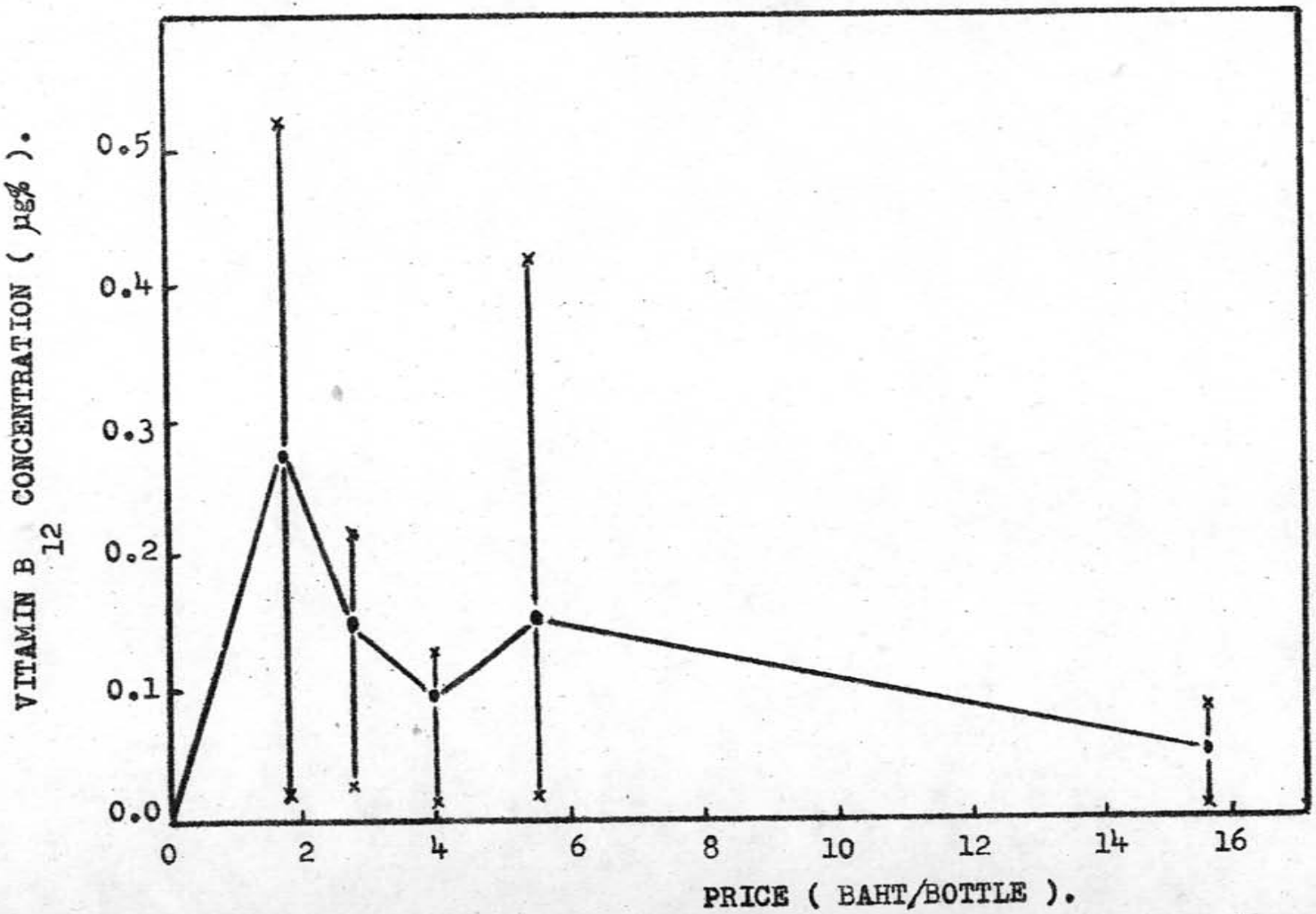

Fig. 5. Relationship between vitamin B_{12} concentration in soya-bean sauce and the price.

3.3 Vitamin B_{12} concentration in fermented fish.

Vitamin B_{12} concentrations were determined from 99 samples of various kinds of fermented fish obtained from markets in Bangkok and other cities of Thailand. These results are shown in Table 6.

Table 6

Vitamin B_{12} concentrations in 99 samples of fermented fish.

Price (Baht/kg)	No.of sample examined	Vitamin B_{12} concentration(µg%)	
		Mean	Range
3.00- 3.50	9	2.28	1.00-3.82
4.00	9	2.78	1.20-6.43
5.00- 6.00	14	3.38	1.23-4.03
8.00	13	3.06	1.27-6.01
9.00	10	2.17	1.49-3.18
10.00	23	2.41	0.94-4.54
12.00-14.00	7	1.77	1.02-2.96
15.00-19.00	6	1.67	0.49-2.94
20.00-25.00	8	0.78	0.33-2.20
Total	99	2.27	0.33-6.43

There was a wide variation of vitamin B_{12} contents in the fermented fish ranging from 0.33 to 6.43 µg % with a mean value of 2.27 µg %. The frequency distribution of these values are shown in Figure 6. There was no relationship between the vitamin B_{12} contents in fermented fish and their prices as shown in Figure 7.

4. Serum vitamin B_{12} level in patients.

4.1 Serum vitamin B_{12} level in patients with Plasmodium falciparum malaria.

The results of serum vitamin B_{12} level in patients with P.falciparum malaria are shown in Table 7. The mean value of serum vitamin B_{12} level estimated in 20 patients was found to be 299 ± 106 pg/ml (range from 121 to 532 pg/ml) which was significantly lower ($p < 0.01$) than the average value of 554 ± 226 pg/ml obtained from 90 control subjects (range 183-1163 pg/ml)

4.2 Serum vitamin B_{12} level in patients with Gnathostomiasis.

The results of vitamin B_{12} level in patients with Gnathostomiasis are shown in Table 8 (See Table 8,P.37). The mean value of serum vitamin B_{12} level estimated in 20 patients was found to be 301 ± 80 pg/ml (range 169-561 pg/ml) which was also significantly lower ($p < 0.01$) than the average value of 554 ± 226 pg/ml obtained from 90 control subjects (range 183-1163 pg/ml)

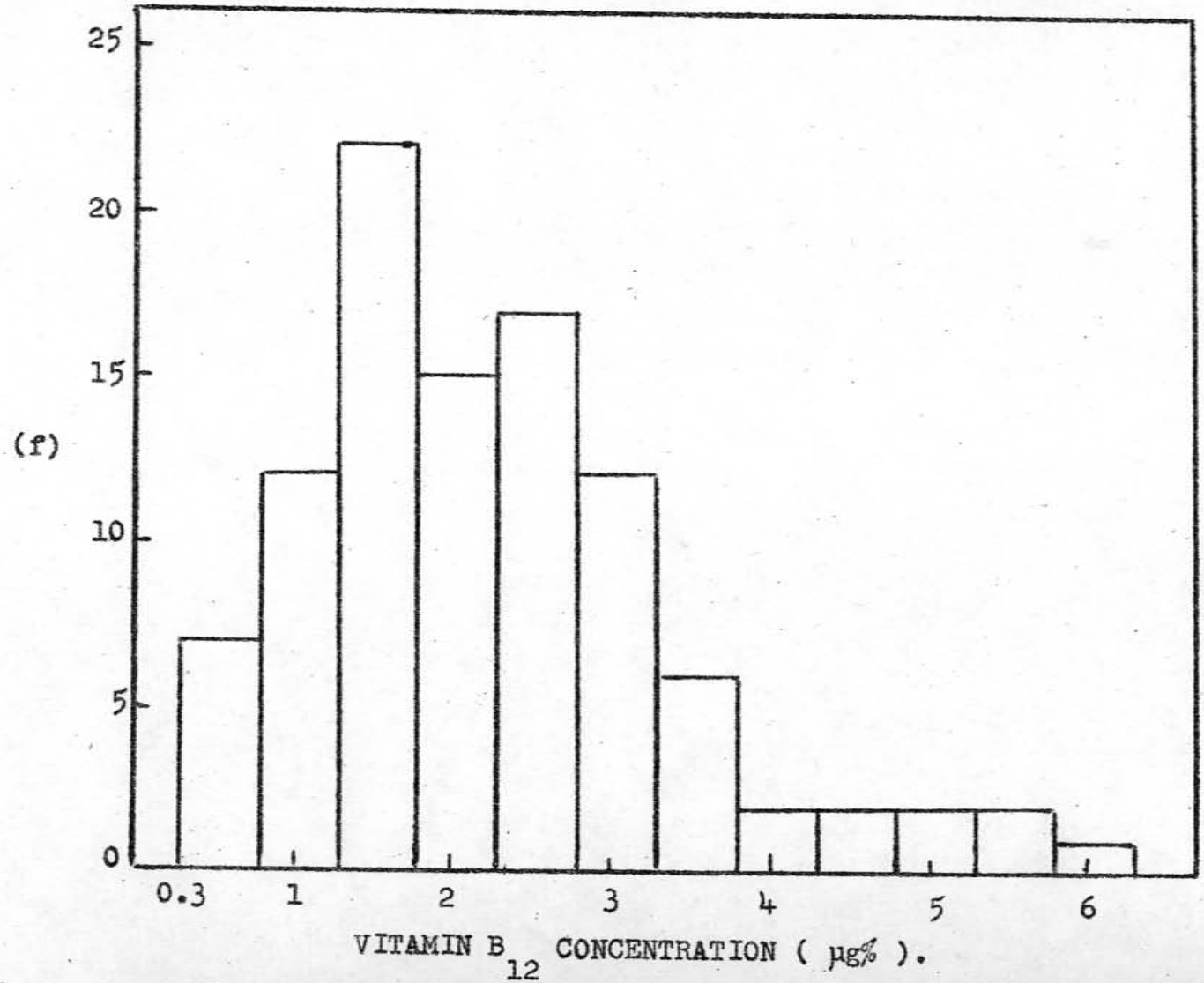

Fig. 6. Frequency distribution of vitamin B_{12} concentration in 99 samples of fermented fish.

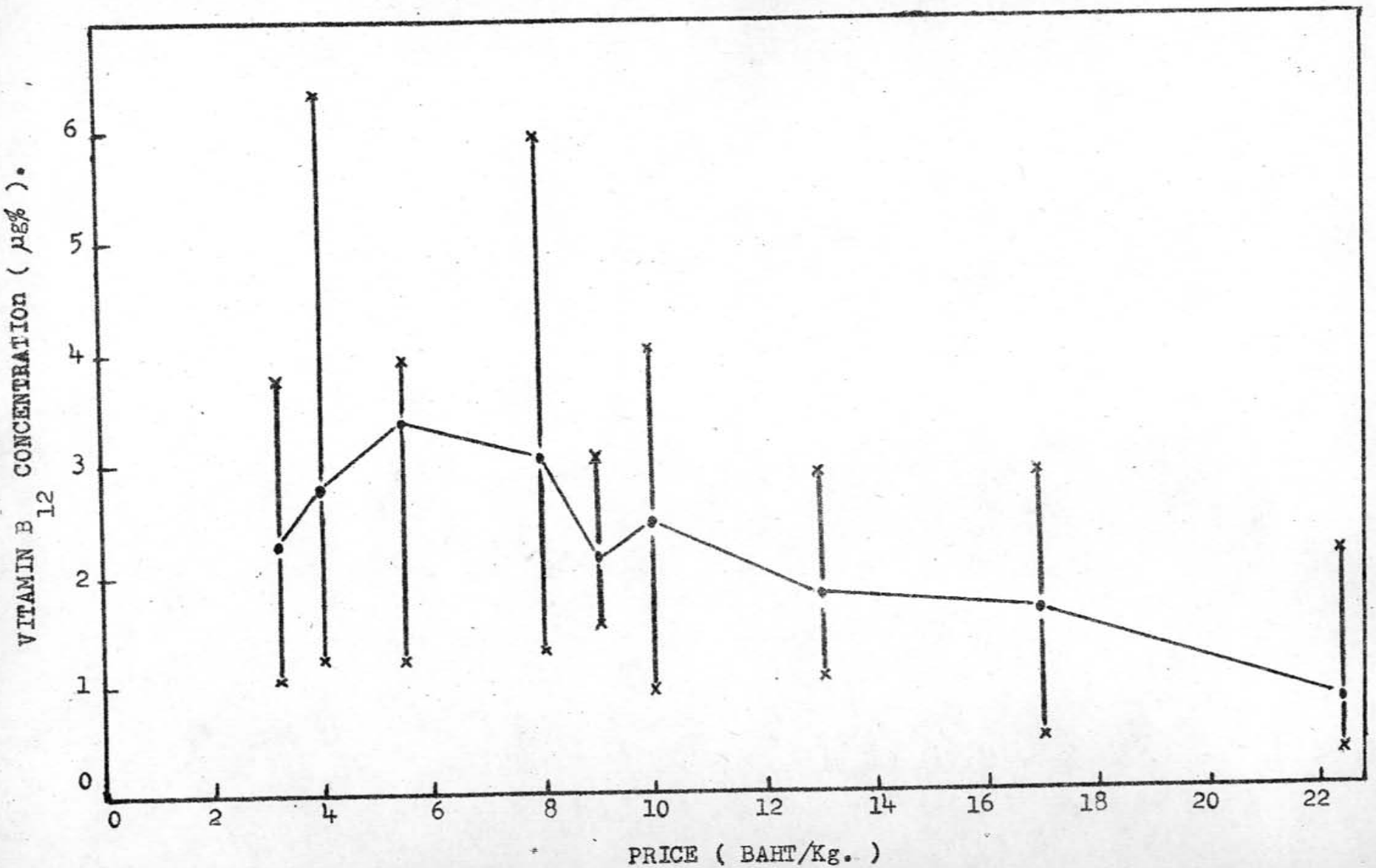

Fig 7. Relationship between vitamin B_{12} concentration in fermented fish and the price.

Table 7

Serum vitamin B_{12} levels in 20 patients with P.falciparum malaria

No.	Subject	Sex	Age(yrs)	Hb (Gm %)	Hct (%)	Serum B_{12} (pg/ml)
1	A	M	22	15.3	46	269
2	B	M	12	8.0	27	219
3	C	M	56	13.3	41	347
4	D	M	16	13.0	40	200
5	E	M	18	8.4	25	152
6	F	M	48	8.7	26	463
7	G	F	64	10.8	34	366
8	H	M	29	15.8	47	532
9	I	M	25	11.2	35	255
10	J	M	21	8.2	25	238
11	K	M	27	12.6	39	356
12	L	M	22	8.0	24	346
13	M	M	23	15.8	47	270
14	N	M	32	13.0	40	449
15	O	M	25	12.2	38	192
16	P	M	33	11.2	35	266
17	Q	M	25	8.2	25	245
18	R	M	21	14.1	43	381
19	S	M	18	9.6	30	121
20	T	M	17	13.3	41	312

N= 20 , $\bar{X} \pm SD = 298.9 \pm 106.4$, Range = 121-532

Table 8

Serum vitamin B_{12} levels in 20 patients with Gnathostomiasis

No.	Subjects	Sex	Age(yrs)	Hb (Gm %)	Hct (%)	Serum B_{12} (pg/ml)
1	A	F	35	12.2	38	382
2	B	M	54	14.1	43	357
3	C	F	10	12.2	38	561
4	D	M	28	17.7	51	212
5	E	M	12	13.3	41	169
6	F	F	23	14.5	44	249
7	G	M	38	14.1	43	309
8	H	M	-	-	-	300
9	I	M	27	13.3	41	298
10	J	M	36	15.0	45	184
11	K	F	25	13.3	41	338
12	L	M	19	15.3	46	334
13	M	M	39	14.5	44	474
14	N	M	32	14.5	44	309
15	O	M	35	14.5	44	412
16	P	M	30	15.3	46	224
17	Q	F	23	14.5	44	200
18	R	M	39	13.7	42	340
19	S	F	52	12.2	38	251
20	T	M	16	15.3	46	230

N = 20 , $\bar{X} \pm SD = 300.5 \pm 80.3$, Range = 184.0 - 474.0

4.3 Serum vitamin B_{12} level in patients with Fasciolopsis buski.

The result of vitamin B_{12} level in patients with F.buski are shown in Table 9.

Table 9

Serum vitamin B_{12} level in 8 patients with Fasciolopsis buski.

No.	Subjects	Sex	Age (yrs)	Hb (gm%)	Hct (%)	Serum B_{12} (pg/ml)
1	A	F	12	14.1	43	200
2	B	M	-	12.2	38	247
3	C	M	-	13.7	42	663
4	D	F	12	13.7	42	315
5	E	F	10	13.7	42	315
6	F	M	9	11.4	36	403
7	G	F	-	-	-	128
8	H	M	-	-	-	689
$N = 8$, $\bar{X} \pm SD = 370 \pm 206.1$ pg/ml , Range = 128-689 pg/ml						

The mean value of serum vitamin B_{12} level estimated in 8 patients was found to be 370 ± 206 pg/ml (range 128-689 pg/ml) which was not significantly difference ($P > 0.05$) from the average value of 554 ± 226 pg/ml obtained from 90 control subjects (range 183 - 1163 pg/ml).

The results of serum vitamin B_{12} level in patients with P.falciparum malaria, Gnathostomiasis and F.buski in comparison with those of the control group are shown in figure 8 (see Fig. 8 , p. 40).

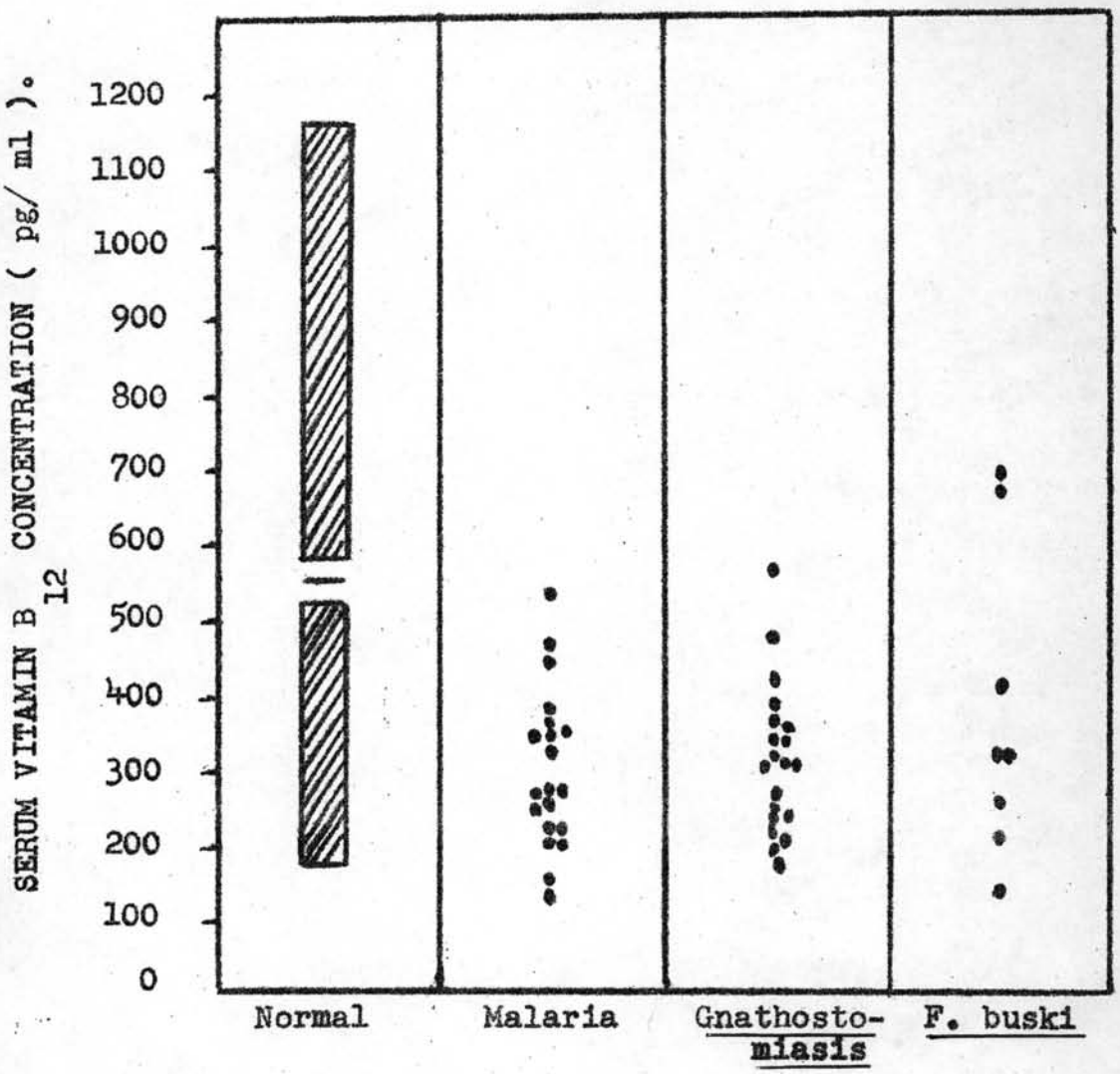

Fig. 8 Serum vitamin B_{12} levels in patients with malaria, Gnathostomiasis and F. buski in comparison with the normal subjects.