particularly in vitamins A and B. The probable factors responsible for the common incidence of the disease in the Circars may be outlined thus:

(*i*) Bad pyorrhœa alveolaris and constipation are so common in these patients that probably the teeth and gastro-intestinal tract act as septic foci from where elective localization of organisms may occur in the stomach or duodenum.

(*ii*) The labourers, the commonest victim of the disease, cannot afford to have regular meals and the irregularity in the intervals and the variety of food may predispose to dyspepsia and frequent pylorospasm. The patient frequently gives a history of such a frequent dyspepsia before the onset of the typical symptoms of peptic ulcer.

(*iii*) The diet of these lower classes is deficient in vitamins A and B, and as McCarrison and others believe such partial avitaminosis probably may give rise to changes in the gastro-intestinal tract which predispose to ulcer formation.

(*iv*) As Tirumurti and Ramachandra Rao (1936) pointed out, 'there is the possibility of malarial infection (Circars abound in agency tracts where malarial infection is very common) favouring the formation of gastric or duodenal ulcers' in at least some of these cases.

Summary

Statistics from the hospital and post-mortem records of the Vizagapatam Medical College Hospital show that peptic ulcer is a fairly common disease in the Northern Circars.

The probable causes for such common incidence are outlined.

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VITAMIN B AND PEPTIC ULCER

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DIET, as a contributory factor in the ætiology of peptic ulcer, was known to the early pathologists. In recent years the subject has been elaborated and various aspects of diet brought to light, and held to be responsible causative agents. Some of them were: 'carried' foods, coarse foods, 'hot' foods, foods at high temperatures, marked difference in the temperature of various dishes; irregular meals, meals at long intervals, etc. That diet probably is partly responsible for the frequency of peptic ulcer in India has been pointed out by McCarrison, Bradfield and Somervell. And the various ways in which the diet was accused were: increased carbohydrate content of the food; markedly low fat diet; gross deficiency of vitamins A, B and C.

Now that laboratory methods have been developed for assaying the various constituents of the food, an investigation into the diet as to its quality and deficiency can be conducted more rationally.

It has been observed by the writer that the diet of the labourer, the common victim of peptic ulcer in South India, is lacking in the essential vitamins A and B, in addition to variations in the proximate principles. The present investigation has been attempted for the purpose of studying the amount of vitamin-B deficiency by microchemical techniques, in patients with peptic ulcer.

Towards the biochemistry of avitaminosis B, contributions have been largely made by the Oxford school of workers. The following may be noted as their essential conclusions. In the brains of experimental polyneuritic pigeons it has been found that there was increased quantity of lactic acid and diminished tissue respiration in glucose or lactate solutions, when compared with healthy controls. It has also been shown beyond doubt that diminished oxygen intake was entirely due to lack of vitamin B in the tissue (Peters, 1936). Hence they concluded that vitamin B was needed for the oxidative removal of lactic acid. Thus the fact was scientifically established that vitamin B was specially concerned with carbohydrate metabolism. Peters and Thompson (1934) have studied the metabolism of pyruvate *in vitro* and came to the conclusion that pyruvic acid, as an intermediary metabolite, was inversely proportional to the vitamin content of the brain. This observation on the brain was applied to other tissues of the body, especially the blood; and it was found that blood has increased intermediate carbohydrate metabolites, especially pyruvic acid which is formed in brain (Thompson and Johnson 1935). Other intermediate carbohydrate metabolites also have been found

in the blood of avitaminous birds—especially methyl glyoxal—as shown by Geiger and Rosenberg (1933), but this could not be corroborated by Gavrilescu and Peters (1931) and Johnson (1936). Johnson and others (1935) attempted to correlate the above facts for the purpose of assessing the vitamin deficiency of an individual by estimating the pyruvic acid in blood. They established the level in normal patients but could find no person with B deficiency in England for investigation. But in China, Platt and Lu (1935) published their observations of the presence of pyruvic acid in the blood of beriberi patients. Subsequently they (1936) confirmed beyond doubt that the same can be applied as a fairly accurate laboratory test for the avitaminosis B.

Many methods have been advocated for estimating the pyruvate in blood. A qualitative one can be used—a modified Rothera's test with nitroprusside and strong ammonia—a blue-green colour indicates the presence of pyruvic acid. Other tests are those of Simon and Piaux's, and Posternak's quoted by Case. Quantitatively various methods of estimation are available.

1. MacLean's method. Precipitation of pyruvic acid with phenyl hydrazine and subsequent estimation of unchanged hydrazine.

2. Clift and Cook's method (1932) is based on the capacity of binding bisulphite.

Each method has its own advantages and disadvantages according to the medium in which the pyruvic acid is contained; namely, muscle filtrate, blood or cerebrospinal fluid.

The two methods used in the present investigation are modified MacLean's and Cook's. The disadvantage of the first method is in the proper extraction of the hydrazone of the pyruvic acid from the blood, the advantage being that pyruvic acid can be estimated as such. Whereas, in the second method the advantage is the amount of accuracy obtained, with the disadvantage that the pyruvic acid is estimated as an unknown quantity of the bisulphite-binding substances in the blood; the proportions of the remaining variable moieties being also unknown. But with the idea of having a clearer reading of the results by a harmonious combination of both the methods it was attempted to use both the methods together and the attempt was only partly successful.

Pyruvic acid.—The estimations were done by Case's method (1932). Peter's and Thompson's modification (1934) was also tried but was found to be less efficient, due to the following reasons :—

1. Large quantities of ethyl acetate have to be used.

2. The extractions are greater in number and not quite satisfactory and demarcative.

3. Greater time is required.

Case's method involves the following principle :—

Excess of 2 : 4 dinitrophenyl hydrazine is added to the deproteinized extract of the blood (the blood being taken under basal conditions). Pyruvic acid hydrazone along with other hydrazones is formed in the solution and with the uncombined hydrazine are extracted with ethyl acetate. Neutralization of the acids used for deproteinization is done to prevent the subsequent extraction of hydrazones other than that of pyruvic acid. Neutralization of the ethyl acetate extract instead of the preliminary trichloracetic acid extract is done to facilitate rapid instead of repeated extractions with ethyl acetate. The combined hydrazones are evaporated and extracted with toluene, which gives them up more readily than ethyl acetate. The pyruvic acid moiety is extracted from the others in toluene with cold sodium carbonate, which dissolves only the pyruvic acid hydrazone in the cold. The 2 : 4 dinitrophenyl hydrazone of pyruvic acid is extracted from the carbonate after acidification with ethyl acetate and crystallized. The estimate is done by the colorimetric method against a standard 2 : 4 dinitrophenyl hydrazone of pyruvic acid, the solvent being alcoholic potash which gives a deep red colour.

Experimental details.—The patient is not permitted to take any solid or liquid food subsequent to the previous night's dinner. The morning coffee was allowed in some cases. Rest in bed is essential in the morning till the time of taking blood, the idea being to eliminate errors due to intermediate products of carbohydrate metabolism, resulting from exercise; 2.8 to 3 c.cm. of blood are taken and immediately transferred to a weighed centrifuge tube containing 7 c.cm. of 5 per cent trichloracetic acid solution, mixed thoroughly, and the exact amount of blood added then obtained by reweighing. After standing for half an hour the precipitate is separated by centrifuging and extracted twice in the same fashion with 5 c.cm. of 5 per cent trichloracetic acid. The combined extracts are then made up to 25 c.cm. and 5 c.cm. are taken and titrated to pH 2.0 with N.NaOH (N/100 HCl with thymol blue acid range is used as control). The amount of N.NaOH required to adjust to pH 2.0 the remaining 20 c.cm. is added and the volume made up to 25 c.cm. with distilled water. One hundred c.cm. are taken out for estimating the bisulphite-binding substances and 15 c.cm. are allowed to stand 24 hours with 0.1 milligramme 2 : 4 dinitrophenyl hydrazine made up in 1 c.cm. of 2 N.HCl. The whole is now shaken with 20 c.cm. of ethyl acetate in a glass-stoppered 50 c.cm. separating funnel. After separation the aqueous layer, which is nearly colourless, is extracted with a further 10 c.cm. of ethyl acetate. As soon as the aqueous layer is colourless, which is usual after three extractions, it is discarded. The united ethyl acetate extracts now contain all the unchanged 2 : 4 dinotrophenyl hydrazine together with the hydrazones which have been formed. The liquid is also acid owing to the extraction of a certain amount of hydrochloric and trichloracetic acids. These are neutralized by shaking with solid calcium carbonate. The solution is decanted into a glass evaporating basin, washing the calcium carbonate with further ethyl acetate until it is colourless. The washings are added to the main bulk of the fluid. The contents of the dish are evaporated on a water bath to 1 c.cm. and then after removal from the bath 20 c.cm. of toluene are added. The slightly cloudy yellow solution is again transferred to the separating funnel and is thoroughly shaken with 5 c.cm. of cold 25 per cent Na_2CO_3 solution. If pyruvic acid is originally present its hydrazone dissolves in the aqueous layer, colouring it brown. This extraction is repeated with fresh Na_2CO_3 solution until the latter remains

colourless. Three repetitions usually suffice. The united sodium carbonate layers are now acidified by adding concentrated hydrochloric acid drop by drop. Great care has to be taken in neutralizing, especially in the last stages, for fear of splashing. If the temperature of the flask rises by rapid addition of acid it is advisable to cool the mixture during the process. The 2 : 4 dinitrophenyl hydrazone of pyruvic acid is precipitated and a lemon-yellow suspension results. This is extracted in a separating funnel with successive portions of ethyl acetate until the aqueous layer is colourless. Usually four 10 c.cm. extractions suffice. The ethyl acetate solution now contains all the pyruvic acid hydrazone which is present and it is evaporated to dryness in a glass basin on a water bath. The yellow residue is dissolved when cool in 5 per cent alcoholic potassium hydroxide, giving a deep red solution which is made up with a further known quantity of alcoholic potash to a colour matchable with the standard.

For the purpose of the standard a pure preparation of pyruvic acid 2 : 4 dinitrophenyl hydrazone is made and a stock solution of this is kept in ethyl acetate of such a strength that 1 c.cm. is equivalent to 0.1 mgm. pyruvic acid. A known volume is taken, evaporated to dryness, and dissolved in alcoholic potash just before actual colorimetry. The standard synthetic 2 : 4 dinitrophenyl hydrazine of pyruvic acid prepared in this laboratory gave a melting point of 216°C. (uncorrected value) at 29°C. temperature and 759.6 mm. pressure, the values given by other authors being 216.5° (Platt and Lu, 1936) and 214° (Allen, 1930).

Bisulphite-binding substance values

Principle.—The deproteinized blood extract at pH 2 is taken and excess of bisulphite added. The excess bisulphite is removed with N/10 and N/100 iodine. Then the combined bisulphite is liberated by the addition of Na_3PO_4 and immediately the amount estimated by N/100 iodine. This is the modification of Clift and Cook's method by Elliott *et al.* (1935).

Experimental details.—The remaining 10 c.cm. of the deproteinized blood extract at pH 2 are taken and allowed to stand for 15 minutes with 2 c.cm. saturated $NaH.SO_3$ solution. One c.cm. starch solution is then added and then the uncombined bisulphite oxidized with N/10 iodine solution till the last drop gives a definite

TABLE I

Serial number	Age	Duration in years	Test meal suggestive of	Barium meal suggestive of	Bisulphite binding substances
1	35	5	Not done	....	5.4
2	27	7	Duodenal ulcer with adhesions.	....	9.3
3	30	6/12	Duodenal ulcer	Irregular and defective filling duodenal cap.	8.6
4	30	..	Normal	....	11.14
5	40	3/12	Cicatrizing duodenal ulcer with adhesions.	....	9.9
6	25	?	Hyperacidity	....	10.12
7	38	1½	Duodenal ulcer	Ill-formed and irregular duodenal cap—ulcer duodenum.	47.3 *
8	35	2/12	Could not be done	Irregular and defective filling duodenal cap.	23.73
9	50	3	Hyperacidity	Hour glass contraction stomach	6.039
10	45	6/12	Hyperacidity	....	6.679
11	25	5	Active duodenal ulcer	Irregular and defective filling duodenal cap.	2.711 †
12	35	7	Duodenal ulcer with stenosis.	A 'niche' in the stomach wall.	1.803
13	55	6/12	Active duodenal ulcer	Defective filling duodenal cap with barium sticking at the ulcer area.	2.097
14	30	2	Nil abnormal	Cap not visualized. At operation showed a duodenal ulcer.	3.115
15	35	'Many'	Duodenal ulcer	Marked pyloric stenosis	2.379
16	40	6/12	Not done	....	5.709
17	40	6/12	Not done	....	6.981
18	25	3	Duodenal ulcer with adhesions.	....	5.709
19	30	3	Active duodenal ulcer	Not definite	4.806
20	50	10	Pyloric stenosis	?	2.897
21	35	3/12	Chronic duodenal ulcer	Cicatrizing duodenal ulcer	5.670
22	30	6/12	Active duodenal ulcer	....	5.568
23	50	15	Duodenal ulcer	Cicatrizing ulcer with pyloric stenosis.	6.714
24	25	4	Not done	Well-formed duodenal cap	3.406
25	55	8/12	Not done	....	5.419
26	50	2	Marked pyloric stenosis	Pyloric stenosis	17.72
27	30	4	Hyperacidity	Barium sticking to the ulcer area in duodenum.	8.939
28	40	6/12	Marked duodenal obstruction.	....	2.801
29	25	2	Duodenal ulcer with stenosis.	Duodenal ulcer	2.716
30	28	2/12	Not done	Barium sticking in the duodenum.	11.58

*Done after meals. †Not done under ideal conditions.

blue colour. The colour of the solution is adjusted to a faint purple, using N/100 Na_2SO_3 solution followed by N/200 iodine solution. Then 2 grammes of $Na_2H.PO_4$—12 H_2O are added and the iodine titration is carried out at once with N/200 iodine made with oxygen-free water. The end point is taken when the standard faint violet persists for 30 seconds. The original method of Clift and Cook required 10 c.cm. of oxygen-free 8 per cent $NaH.CO_3$ solution instead of the phosphate and carrying on the final titration at low temperatures. The bisulphite binding substances are expressed as mgm. Pyruvic acid per 100 grammes fluid (1 c.cm. N/100 iodine—0.44 mgm. pyruvic acid).

Results.—Table I summarizes the findings of bisulphite-binding substances in the blood of patients suffering from gastric or duodenal ulcers the diagnosis being made by history, test meal and barium meal findings. Some of them that were operated upon were verified.

The pyruvic acid as hydrazone is estimated in nearly all the cases but unfortunately values of many of the cases had to be discarded because of the slight difference in the tint for calorimetric estimation which usually crept in on account of two reasons, *viz*, the least delay in calorimetry after the development of the colours and impurities in the ethyl acetate with which the pyruvic acid is extracted.

Table II shows the pyruvic acid values when estimated as pyruvic acid dinitrophenyl hydrazones.

TABLE II

Serial number	Bisulphite-binding substances	Pyruvic acid	Percentage
1	5.4	1.16	*
2	9.3	1.441	*
3	8.6	6.761	78.67
4	11.14	1.982	17.81
5	9.9	3.685	*
6	10.12	3.118	*
7	47.03	0.117	*
8	23.73	0.619	2.61
9	6.7	5.969	78.48
10	2.7	0.571	*
11	1.8	0.873	48.51
12	2.1	0.417	20.33
13	3.1	2.178	70.26
14	5.99	4.933	82.35
15	6.98	1.253	17.95
16	5.7	0.644	*
17	4.8	2.722	56.71
18	2.9	2.845	99.64
19	5.67	3.798	84.31
20	5.57	5.388	96.72
21	6.67	6.362	94.95

* Indicates a greater percentage of experimental error.

Comment

It is difficult to assess the amount of pyruvic acid present in man during health. The only record that could be found is that of Platt and Lu (1936). They have recorded the readings of 23 healthy Chinese college students whose values varied from 2.22 to 4.82 mgm. bisulphite-binding substances per 100 gm. of blood with an average of 3.27 mgm. An attempt has been made to find the bisulphite-binding substances values in healthy labour classes, as the majority of the 30 cases studied is from the same classes.

TABLE III

Serial number	Values of bisulphite-binding substances
1	4.7
2	3.1
3	2.7 *
4	5.2 *
5	3.3
6	3.1
7	6.353
8	4.580
9	3.228
10	3.906
11	1.704
12	3.354
13	1.457

* These cases could not be studied under basal conditions.

Thus it is seen that considering the 11 cases done under ideal conditions the bisulphite-binding substances values vary from 1.457 to a maximum of 6.353, the average being 3.457 mgm. Platt and Lu report the content of the blood in established beriberi cases to be markedly increased. But in none of the cases have they reported values higher than 10 mgm. per cent. But in this connection it must be mentioned that some of the 'gastric' cases showed values higher than 10. An attempt has been made to find the corresponding values of beriberi cases admitted to the hospital. Seven cases showing marked avitaminosis B were studied and the values are noted hereunder (table IV).

TABLE IV

Serial number	Values
1	9.4
2	7.7
3	7.7
4	4.3
5	6.7
6	15.08
7	4.91

It is interesting to note that one of the cases (6) has shown a value of above 15, and others, except case 4, show uniformly high values.

Of the bisulphite-binding substances content of 'peptic ulcer' patients excluding cases 7 and 12, where there was much unavoidable experimental error, the variation is from 2.1 to a maximum of 23.73.

Fixing the maximum in a normal individual as 4.8, as judged from the healthy students, nine of the values are within the normal range and the remaining ones beyond it; so much so that it appears that higher bisulphite-binding substances values are fairly frequent in

these patients. But this observation is not without possibilities of error, the chief of which is the presumption that the pyruvic acid moiety of the bisulphite-binding substances always bears the same high percentage to the total. But from the readings in Table II it appears it is not quite true. The observations on this aspect are so meagre that the discussion on the subject cannot be complete, but from the proof advanced so far by Platt and Lu (1936) that bisulphite-binding substances values run parallel to the vitamin-B deficiency it may be concluded with a fair amount of certainty that these patients are suffering from partial avitaminosis B. The truth of the statement may be substantiated by the fact that it is in these lower classes that beriberi is fairly common.

The discussion of the subject on the basis of these findings and findings of animal experiments will be given in another paper.

Summary

Bisulphite-binding substances in blood of peptic ulcer patients are found to be increased in a good percentage of cases, showing a definite deficiency of vitamin B in these patients.

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TREATMENT OF PHRYNODERMA BY VITAMIN-A CONCENTRATE

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Phrynoderma (toad-skin) is the name given to a papulo-follicular dermatosis commonly seen in malnourished individuals (Nicholls, 1933). During recent years considerable attention has been paid by nutrition workers to the study of the condition. A brief review of the literature on the subject and an account of its clinical and anatomical features have been given in a previous communication (Radhakrishna Rao, 1937*a*).

While it is generally agreed that phrynoderma is a separate clinical entity associated with malnutritional states, the exact cause of the condition is still not quite clear. Several workers have described the condition as occurring in association with xerophthalmia and keratomalacia. This finding, together with the results of the histological examination of the papular eruption in phrynoderma, suggested that the condition was probably attributable to a deficiency of vitamin A in the diet. The beneficial results, reported by several workers, of the therapeutic administration of cod-liver oil and, in a few cases, of vitamin-A concentrate on the papular eruption support the above view. Frazier and Hu (1931, 1935 and 1936) have observed that as a result of providing a well-balanced diet and administering 30 c.cm. of cod-liver oil daily, and without any local medication, the papular lesions gradually disappeared, leaving delicate, atrophic, pigmented scars. Loewenthal (1933) also reports the beneficial effects of the administration of cod-liver oil and, in two cases, of 'avoleum'—an extract containing only vitamin A. Reiss (1936) and Youmans (1937) obtained similar results after the administration of cod-liver oil. Goodwin (1934) reported a case in London in which the papular eruption disappeared when the patient was given a good mixed diet with the addition of cod-liver oil. Frazier and Hu (1936) also tried the effect of treatment with carotene (subcutaneously) in one case and halibut-liver oil in another with equally satisfactory results. Nicholls (1935) mentioned that milk or eggs quickly cured phrynoderma and 'sore-mouth'.

This paper reports the results of a clinical investigation of the effect of vitamin-A concentrate on the papular eruption in phrynoderma.

Description of cases and results of treatment

The investigation, of which the present paper forms a part, was commenced in a children's boarding school in the Nilgiris, and later on extended to a day school in the same district. The majority of children attending these schools belong to poorer classes. Children who showed marked evidence of phrynoderma were