TABLE I.
Effect of Growth Factors upon Development of Several *Pasteurella* Species of Animal Origin.

Basal medium with addition of:	Amt added, μg per cc of medium	*Pasteurella* avicida days* 1	2	boviseptica I days 1	2	boviseptica 18 days 1	2
Nothing (control)	—	—	—	—	—	—	—
Nicotinamide	0.1	—	—	—	—	—	—
Pantothenic acid	0.1	—	—	—	—	—	—
Nicotinamide plus pantothenic acid	0.1 each	+++	+++	+++	+++	±	+
Nicotinamide plus beta-alanine	0.1 each	—	—	—	—	—	—
Butyl factor	0.15	—	—	—	—	—	—
Nicotinamide plus pantothenic acid plus butyl factor	0.1 0.1 0.15	+++	+++	+++	+++	++	+++

— = No visible growth, ± = very light turbidity just at point of visibility, + to +++ = light to pronounced turbidity.
*All cultures were held for 10 days at 37° and observed at frequent intervals. Usually there was no change after the second day.

3 of the remaining 4 cultures. The growth of *P. boviseptica* 18 (Table I) is an example of the effect of addition of butyl factor.

On substitution of a mixture of 18 amino acids for the hydrolyzed gelatin solution it was found that with but two exceptions all of the cultures could be grown in the presence of nicotinamide, pantothenic acid and the butyl factor. As far as we are aware cultivation of these organisms in an amino acid medium with the addition of known accessory substances has not hitherto been accomplished.

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Normal and Abnormal Prothrombin Levels.

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While low fibrinogen levels have been reported in pernicious anemia, scurvy, pellagra, acute yellow atrophy, and myeloid dyscrasias, there are not available adequate data as to the prothrombin levels of such diseases in man. Quick[1] postulates that the blood in acute yellow atrophy of the liver may be as deficient in

[1] Quick, A. J., *Am. J. Med. Sci.*, 1940, **199**, 123.

prothrombin as in fibrinogen, and regards it highly improbable that a prothrombin deficiency can be produced in the adult by dietary means. It must be remembered that pellagra and sprue are both commonly associated with rather characteristic dietary habits, and evidence has already been offered for prothrombin deficiency in sprue,[2] and that for hypoprothrombinemia in the absence of jaundice in man due to inadequate vitamin K intake.[3] Likewise, there exist bone marrow and intestinal relationships in sprue, pernicious anemia and pellagra.

The present report was undertaken on a small series of cases to determine the average normal limits of prothrombin clotting time in normal adult subjects and in treated and untreated patients suffering from unrelated diseases. The amount of prothrombin was estimated by the methods of Quick[4, 5] and Warner,[6] and compared with the prothrombin index of whole blood. A total of 94 clinic and hospital patients, and 17 adult normals were observed over a period of 3 months. Six tests were performed on each concentration of 10% and 5% thromboplastin, with blood taken from the median basilic vein. The potency of the thromboplastin was estimated on the normal adults and was carefully prepared. The data are expressed in the number of seconds required for the clot formation to appear at 37°C with each lot of freshly prepared thromboplastin.

It has been shown by previous investigators that the normal prothrombin time is 16 to 19,[4] 20,[8] and 25[7] sec. with an error of only 5%. Warner, *et al.*, with a plasma dilution method to evaluate the deficiency in prothrombin units have an approximate variation of less than 5%. It seems significant to compare the 3 tests for an index of normal with fresh blood and plasma under ideal conditions.

Table I illustrates the average values for prothrombin clotting time in normal and pathologic patients.

Patients with undiagnosed blood dyscrasias were omitted from the final series. Although the experimental error in the series by the wet and dry methods occasionally exceeded 5-10%, the results none-

2 Clark, R. X., Dixon, C. F., Butt, H. R., and Snell, A. M., *Proc. Staff Meet. Mayo Clin.*, 1939, **14**, 107.

3 Kark, R., and Lozner, E. L., *Lancet*, 1939, **2**, 1162.

4 Quick, A. J., *Am. J. Med. Sci.*, 1935, **190**, 501.

5 Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

6 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

7 Aggeler, P. M., and Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 11.

8 Kato, K., and Poncher, H., *J. A. M. A.*, 1940, **114**, 9, 749.

TABLE I.

No. of cases	Diagnosis	Sec. with thromboplastin 10% Wet	5% Dry	Whole blood index	% of normal (Warner)
8	Sprue	38	30	1.0	88
2	Thrombocytopenic purpura	39	32	1+	80
6	Polycythemia	29	30	1.0	86
3	Microcytosis	28	25	1.0	92
5	Acute pernicious anemia (untreated)	30	23	1.0	96
12	Pernicious anemia with cord lesions	27	25	1.0	90
16	Pernicious anemia without cord lesions (treated)	27	30	1.0	82
9	Alcoholic polyneuritis	33	30	1+	82
1	Sprue (relapsing)	60	31	1+	76
1	Parathyroidectomy (under treatment)	20	29	1.0	100
4	Pellagra with alcoholism	29	26	1.0	94
3	Tabes with malaria	30	30	1.0	86
1	Partial gastrectomy (Ca)	28	32	1+	82
5	CNS Lues with malaria	32	28	1.0	89
3	CNS Lues without malaria	30	32	1+	84
4	Sickle cell anemia	23	19	1.0	92
1	Lymphogranuloma inguinale	26	21	1.0	96
3	Aplastic anemia	30	33	1+	78
2	Diabetes mellitus	25	27	1.0	90
1	Splenectomy	34	30	1.0	86
2	Hodgkins Disease	32	29	1.0	86
2	Brucellosis	32	26	1.0	92
17	Normal	26	28	1.0	94

theless, under the ideal conditions show that the clotting time of prothrombin in the normal adult ranges up to 30 sec. as the upper limit with a prothrombin index of 1.0 for whole blood clotting. If 25-30 sec. is taken as the upper limit of normal, closer correlation may be found to exist with the other tests. The recent report of Kato and Poncher using the microprothrombin test of Kato[8] on mature and immature infants, shows that the most mature infants likewise fall in the 25-30 sec. range. This seems to substantiate the results reported here.

At times it is difficult to account for the high readings given by dry thromboplastin, and low readings or *vice versa* from the wet solution prepared from the same source, with more uniform results occurring in this series with the dried material, if the only variable factor is the quality and amount of the prothrombin of the test plasma. Comparison of the prothrombin index of whole blood yields correlation to the studies reported here. Grossly abnormal tests if repeated will establish the general average range which has been observed to be 25 sec. plus or minus in the pathologic cases reported here and not above 30 sec.

None of the patients received synthetic vitamin K in therapy

and depended upon its content in their food. Nicotinic acid and vitamins B_1 and riboflavin were given to the pellagra patients. Alcohol-polyneuritis patients received vitamins B_1 and riboflavin and adequate food.

The sprue patients are interesting, since none of them received synthetic vitamin K and maintained adequate prothrombin levels. A case of sprue (relapsing) with marked diarrhea showed an almost normal prothrombin level. The sprue cases received liver extract and yeast as did all pernicious anemias.

Despite the fact that prolonged readings have been observed in individual cases of pernicious anemias the general average reveals no marked deficiency of prothrombin. This apparently holds true for pellagra and polycythemia, treated and untreated. In a case of aplastic anemia following anti-luetic therapy, the prothrombin level was slightly reduced. While none of these patients enjoy maximum health, it is significant that an approximately normal or slight diminution of the prothrombin level is found even during characteristic exacerbations of their diseases. The whole blood clotting index may even be less than 1.0, illustrating that the volume per volume content of prothrombin in whole blood and plasma is not very significant.

Conclusions. (1) From the data presented the normal range of prothrombin-clotting time has been observed to be from 25 to 30 sec. Values above 30 sec have given a whole blood index of 1.0 plus. These results yield closer correlation to other methods for estimating the prothrombin level. (2) Treated and untreated patients with pernicious anemia, pellagra and other pathologic conditions have similar normal prothrombin values. Treated sprue patients gave similar results. In a case of sprue (relapsing) with persistent diarrhea, the prothrombin level was found to be almost normal. (3) When abnormal variations occur in wet or dry readings, it may be more accurate to depend upon the whole blood clotting index.