analysis of p-hydroxaminobenzenesulfonamide are described. When injected into dogs, this substance appears to be completely converted to sulfanilamide within 5 minutes. *In vitro,* under the conditions of our experiments, it is no more than ten times as active as sulfanilamide.

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Vitamin K Deficiency and Prothrombin Levels. Effect of Vitamin K Administration.*

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In using the chick for the assay of vitamin K, certain workers have used the preventive technic,[1] and thus have given an indication of the basal need of the chick for this vitamin. Others have used the curative technic. Ansbacher[2] has used a curative period of 6 hours, and Thayer and coworkers[3] have lengthened the period to 18 hours. With periods longer than this,[4] the basal utilization becomes significant, we believe, and the assay represents a combination of preventive and curative technics.

Both the preventive and the curative technics supply incidental information concerning physiological problems of great importance. Unfortunately, those who have used brief curative periods have not supplied data on the prothrombin level, but have simply used the whole blood clotting time as a measure of deficiency and of response to treatment. It is our present purpose to give part of the data which are still lacking. It is hoped that these data will help to clarify the various assay procedures now in use, and at the same time provide a much needed quantitative correlation between the prothrombin level and the vitamin K supply.

Materials and Methods. White Leghorn chicks, newly hatched,

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1 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition,* 1937, **14**, 235.

2 Ansbacher, S., *J. Nutrition,* 1939, **17**, 303.

3 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

4 Dam, H., and Glavind, J., *Biochem. J.,* 1938, **32**, 1018.

were placed on Almquist's diet,[5] modified to contain 2.5% of the salt mixture of Hubbell, Mendel and Wakeman. The salt mixture was also supplemented with 0.2% each of $MnCl_2$, ferric citrate and $AlCl_3$.[6] The ground rice was extracted several days with ether; the fish meal and brewer's yeast were extracted for 10 days in a giant Soxhlet extractor. To prevent bacterial action, the diet was stored at −40°. A control diet, on which normal prothrombin values are based, was similar, but the rice content was decreased sufficiently to permit inclusion of 10% alfalfa meal in the diet. Special care was taken to prevent coprophagy.

Blood for clotting times was obtained by brachial vein incision. Blood (1 cc) for prothrombin determinations was obtained by cardiac puncture, using a 1 cc tuberculin syringe containing 0.2 cc of 1.85% potassium oxalate. The blood was centrifugalized in a special tube and the hematocrit noted. In conducting the 2-stage titration procedure for prothrombin,[7, 8] it was found difficult to defibrinate the plasma completely with thrombin. To make this possible, the plasma was diluted 3-fold with saline as a preliminary measure. In making the prothrombin calculations, a correction was made for this dilution.

Results. The plasma of newly-hatched chicks contains approximately 58 units of prothrombin per cc (Fig. 1). In contrast to this, we have found that the plasma of adult roosters contains approximately 175 units per cc.[9] Work from our laboratory[10, 11] has already shown that the prothrombin level in human infants is likewise about 30% of normal. In both cases the results were obtained with a 2-stage titration technic in which complete conversion of the prothrombin into thrombin is followed by titration of the latter. We believe that the total amount of thrombin which can be formed is the best index of the amount of prothrombin present. The high values obtained in baby chicks by other workers[12] may well be due to the use of prothrombin tests which are based primarily upon prothrombin conversion rate.[11]

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5 Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

6 Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273; Wilgus, H. S., Jr., Norris, L. C., and Heuser, G. F., *J. Nutrition*, 1937, **14**, 155.

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

8 Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

9 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296.

10 Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. Med. Sci.*, 1937, **193**, 475.

11 Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 181.

12 Schønheyder, F., *Am. J. Physiol.*, 1938, **123**, 349.

The 58 unit prothrombin level of newly hatched chicks is an average obtained from the study of many chicks. Inspection of individual protocols indicates that analyses made during the summer months tend to be at least 20% higher than those made in the late fall. It has been suggested by others[13, 3] that the storage of vitamin K in the egg, and the rapidity with which chicks become deficient when on a vitamin K-free diet may vary in different seasons because of differences in diet supplied the laying hens. As far as we are aware, our observation is the first to suggest that the prothrombin level at time of hatching may have a corresponding variation.

When the chicks were placed on the K-deficient diet a gradual fall in the plasma prothrombin level began almost at once (Fig. 1). The fall was not quite so rapid, however, as between the 3rd and 6th days of life. It is evident, nevertheless, that the reserves of vitamin K, carried over from the egg, are not great. The chicks hatched during the summer months maintained their prothrombin levels a day or two longer than those hatched in the late fall, indicating, again, somewhat greater storage of vitamin K in the former.

At about the 7th day of life the clotting time of whole blood becomes prolonged, and at that time the plasma prothrombin level was found to be approximately 30% of normal for chicks of the same age. The variability of clotting time is considerable, however, and the results do not reflect accurately the changes in prothrombin level.

During the next few days the prothrombin level fell slightly more,

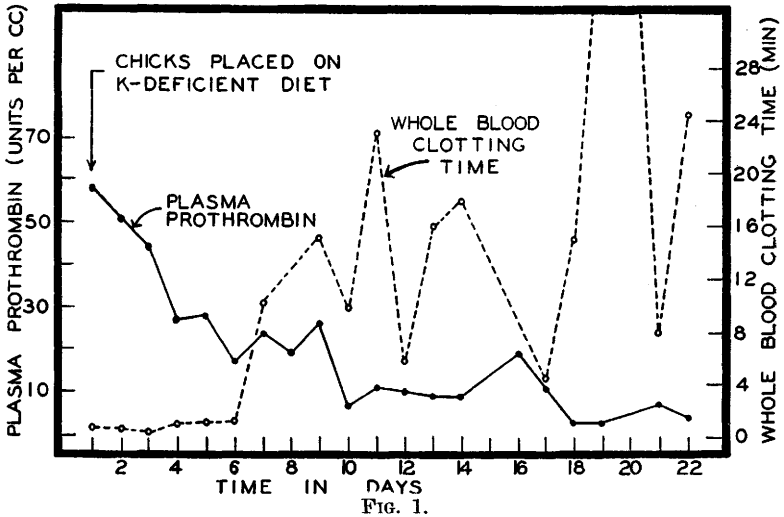

FIG. 1.
Prothrombin Depletion Curve.

[13] Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

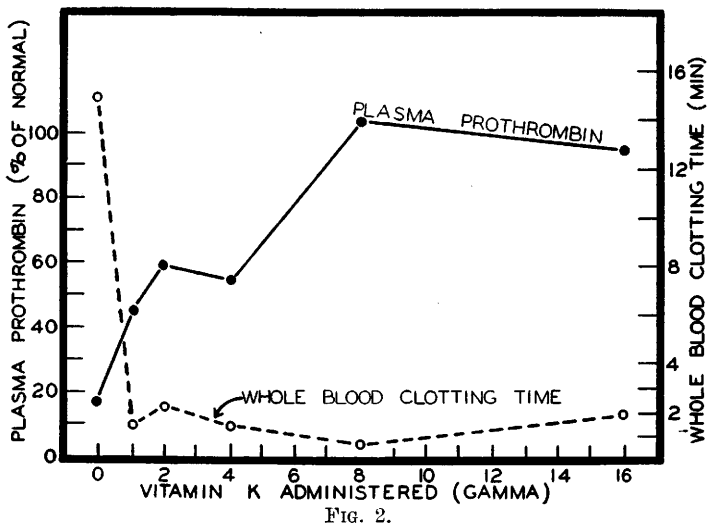

FIG. 2.
Prothrombin Levels. Effect of Vitamin K.

and a number of the chicks showed spontaneous hemorrhages. It is evident that the bleeding level is reached when the prothrombin falls to approximately 10% of the values seen in normal chicks of the same age.

Fig. 2 shows the effect of single doses of vitamin K in effecting cures in deficient chicks. Varying doses of 2-methyl-1,4-naphthoquinone (1.0-16.0 gamma in 0.2 cc corn oil) were placed directly in the crop. After the lapse of 18 hours the clotting time and the prothrombin level were determined. Doses of 8 gamma, or more, uniformly brought the prothrombin level almost completely to normal in this length of time. Smaller doses had progressively less effect, though a definite rise was still evident on administration of as little as 1.0 gamma of the compound.

The question at once arises as to whether an 18-hour period is sufficiently long to permit the maximum effect of the vitamin to become manifest. Our data on this point are still incomplete, but evidence indicates that 18 hours produces almost as much effect as 42 hours, even in cases where the dose is inadequate to effect complete recovery.

When the recovery period is shortened to 6 hours, recovery was again found to be practically complete in case the dose was 16 gamma or more. With smaller doses this is not the case. We have performed a number of experiments at the 8 gamma level, and have found that after 6 hours the prothrombin recovery was only 50% complete.

The whole blood clotting time, prolonged in the deficient chicks prior to treatment, returned to normal after the chicks were treated with 1 gamma of the vitamin. The prothrombin level was still less than one-half of its normal value, however. It is thus evident that those vitamin K assay technics which are based upon whole blood clotting time do not indicate the amount of vitamin needed to effect a complete cure. At most, they indicate approximately the amount of the vitamin needed to bring the prothrombin above the 30% level.

The use of the 6-hour curative period in assay work introduces a second factor which has not been recognized, *i. e.,* the fact that adequate time is not allowed for the maximum action of the vitamin. If the 6-hour principle is combined with the use of whole blood clotting time as an index of deficiency, it is evident that the two variables tend to neutralize each other, though to an unpredictable degree.

Much of the assay work to date has been done for the purpose of comparing two or more products of unknown potency. In dealing in this way with relative values, it is probable that any one of the various technics reported would give comparable data. Any effort, however, to establish absolute biological units should take into account the facts which we have outlined.

The fact that large doses effect recovery more rapidly than smaller ones has important implications for the clinician. It is often a matter of great importance that the bleeding tendency seen in vitamin K deficiency be eliminated with minimum delay. When the liver is impaired, or the body has for other reasons a decreased ability to form prothrombin, it is possible that large doses of vitamin K will permit more effective use of whatever capacity may remain for the production of prothrombin. This concept is consistent with the fact that some patients require far more vitamin K than others.

Summary. A detailed study was made concerning the rate at which the plasma prothrombin level falls when newly hatched chicks are placed on a vitamin K-deficient diet. The whole blood clotting time becomes prolonged when the prothrombin level falls to about 30% of the normal level for chicks of the same age. Hemorrhages make their appearance when the prothrombin is approximately 10% of normal.

It was also shown that large doses of vitamin K correct the plasma prothrombin deficit almost completely within 6 hours. Somewhat smaller doses effect partial recovery in 6 hours, and almost complete recovery in 18 hours.

Questions of assay technic and of therapeutic needs are discussed.