Relationship of Vit. A and Carotene to Bovine Serum Proteins.*† (24631)

E. S. ERWIN, T. R. VARNELL AND H. M. PAGE (Introduced by A. R. Kemmerer)
Dept. of Animal Science, University of Arizona, Tucson

The relationship of circulating blood carotenoids to serum proteins was recognized in 1914 by Palmer and Ekles(1). More recently, specificity of the affinity of certain serum and lipoproteins for both Vit. A and carotene have been investigated. In human serum, Crook and El Marsafy(2) reported that carotene was primarily associated with beta globulin and albumin fractions when serum proteins were separated electrophoretically, while Krinsky, *et al.*(4) using different technics found that 90% of circulating carotenoids were bound to alpha and beta lipoproteins. However, these lipoproteins bound only a small fraction of circulating Vit. A. Using ammonium sulfate fractionations of human serum proteins, Dzialoszynski, *et al.*(3) concluded that Vit. A and carotene were associated with albumin. A hypothesis has been advanced that binding capacity of serum proteins aids in thwarting electrolytic destruction of Vit. A and carotene. The quality of dietary protein for rats has been shown to alter utilization of dietary carotene(5). This phenomenon may have been mediated through alteration of type and quantity of protein in serum. This investigation was initiated to elucidate association of carotene and Vit. A to specific serum proteins in the bovine animal. Further, alteration of serum proteins by Vit. A deficiency was studied.

Method. In vitro. Proteins from 30 ml of serum, from a Guernsey cow, were separated in Spinco continuous-flow electrophoresis apparatus into 16 fractions at room temperature (75°F) at the rate of 1 ml/hour with sodium veronal buffer (pH = 8.6; u = .02) (90 ma; 1000 volts). Electrophoretic mobility of lyophilized protein fractions on paper strips was compared to original serum sample. Vit. A and carotene were extracted in light petroleum ether from protein fractions previously treated with ethanol. Because of apparent loss of Vit. A, a second electrophoretic separation of serum proteins, from the same cow, was performed in a dark refrigerated room (36°F). The procedure differed only in amount of current applied (60 ma; 650 volts). Protein fractions were saponified in alcoholic KOH prior to extraction of Vit. A and carotene in light petroleum. Identification and relative concentration of carotene in the protein fractions were performed by spectrophotometric analyses while Vit. A was determined by Carr Price reaction.

In vivo. Two identical twin Holstein heifers, approximately 20 months old, were fed carotene-free ration of straw, grain, and cottonseed meal until Sept. - (150 days). One million I.U. of Vit. A were administered orally *via* capsule to animal "B". At this time electrophoretic patterns of serum proteins in both heifers were similar. On Sept. 23, animal "A" exhibited acute Vit. A deficiency syndromes manifested by blindness in one eye and anasarca in both fore and hind legs. Blood was withdrawn from both heifers for electrophoretic separations of plasma and serum proteins 3 days following onset of deficiency syndromes. An aqueous emulsion of beta carotene was prepared with Tween 80 (3 mg/ml) and intravenously administered to both heifers in accordance with body weight (10 mg/100 lb). Blood was withdrawn prior to and at 5 minutes, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 hours following carotene administration for plasma carotene and Vit. A analyses(6). Five days following carotene injection, serum and plasma from both heifers were subjected to electrophoretic separations of proteins. An aqueous emulsion of Tween 80 and Vit. A acetate was intravenously injected into both heifers(10 mg/100 lb body weight). Blood samples were withdrawn at 4 and 10 days following, and plasma and serum proteins were separated electrophoretically. Electrophoretic separations of plasma and serum proteins of the heifers were per-

* Scientific Paper No. 501. Arizona Agri. Exp. Station, Tucson.

† This investigation was supported in part by U.S.P.H.S. research grant.

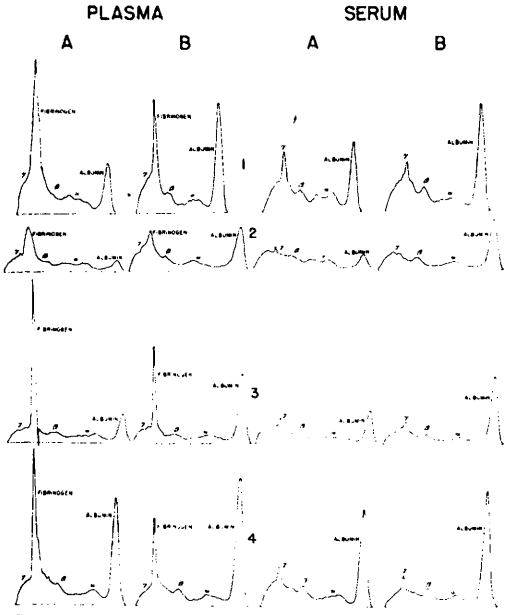

FIG. 1. Alteration of plasma and serum protein fractions as influenced by vit. A deficiency. A = deficient; B = normal. No. 1 and 2 = progressive vit. A deficiency; No. 3 and 4 = recovery following vit. A administration.

formed on paper strips (0.006 ml) in Durrum type cell with sodium veronal buffer (pH = 8.6; u = .075) for 14 to 16 hours.

Results. In vitro. Initially, bovine serum proteins were electrophoretically fractionated at room temperature. Carotene was associated with bovine serum proteins in the following percentages: albumin, 75.1; alpha globulin, 6.8; beta globulin, 8.9; and gamma globulin, 9.2. Vit. A could be detected only on albumin. Much of Vit. A and carotene was degradated during fractionation. Therefore, the second elctrophoretic separation of serum was performed in a dark refrigerated room. The low temperature necessitated use of less current (60 ma). This fractionation resulted in undefined separation of albumin and alpha globulin. All protein fractions were saponified and Vit. A and carotene were extracted in light petroleum. Only 2% of total carotene was found in fractions other than albumin and alpha globulin. While no Vit. A was associated with gamma globulin, beta globulin possessed 15% and albumin-alpha globulin fraction contained 85% of total amount of serum vitamin. These results support the evidence of others such as Ganguly, *et al.*(7), that Vit. A and carotene are bound to specific proteins in serum. *In vivo.* Growth patterns, hair swirls, color markings and blood typing were used to establish that heifers were identical twins. Further, electrophoretic separations of serum protein of both animals on Sept. 1 were similar. On Sept. 26, three days following onset of acute Vit. A deficiency syndromes in animal "A" (blindness and anasarca) serum albumin in the deficient heifer was lowered (Fig. 1, No. 1). Since our previous *in vitro* investigation revealed that albumin bound the majority of circulating carotene in sera, similar amounts of carotene (based on body weight) were intravenously administered to both deficient "A" and normal "B" heifers. Disappearance rates of plasma carotene revealed that the deficient animal maintained approximately half the plasma carotene level of the normal heifer(Fig. 2). Probably this phenomenon was related to low serum albumin level. Ganguly, *et al.*(7) hypothesized that specific serum proteins may be responsible for absorption of Vit. A and carotene. This thesis would lend support to recent study by Diven and Erwin(8) which indicated that Vit. A-deficient sheep lost in part the ability to utilize dietary carotene. Plasma Vit. A analyses substantiated the findings of Church, *et al.*(9), that the mature bovine was unable to convert metabolic carotene to Vit. A. On Oct. 2, electrophoretic separation of plasma and serum protein (Fig. 1, No. 2) showed that albumin content was further depressed. Thus, metabolic carotene did not alleviate ab-

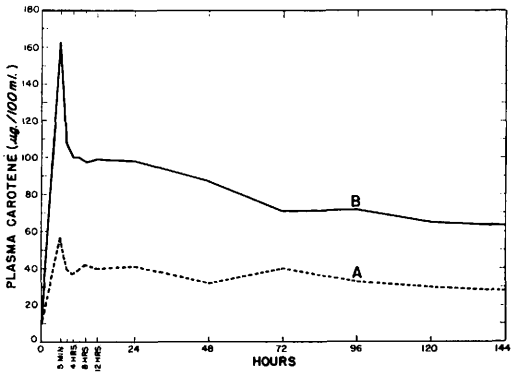

FIG. 2. Effect of vit. A deficiency on carotene capacity of bovine plasma. A = deficient heifer; B = normal heifer.

normal serum protein patterns. Four days following intravenous Vit. A acetate administration, Vit. A deficiency syndromes in heifer "A" disappeared and albumin content of sera was elevated (Fig. 1, No. 3). Electrophoretic separations of sera 6 days later (Fig. 1, No. 4) showed elevation in serum and plasma albumin that approximated normality. These results further exemplify that metabolic carotene in the bovine possesses little value in alleviating syndromes predisposed by Vit. A deficiency.

Summary. Bovine serum protein was fractionated with a continuous flow electrophoresis apparatus. Vit. A and carotene were principally associated with albumin. When one co-twin of identical twin heifers was subjected to Vit. A deficiency, serum albumin was progressively reduced. Following an intravenous injection of carotene, the deficient heifer low in serum albumin retained approximately one-half the plasma-carotene concentration as her normal co-twin. Serum albumin returned to normal in the deficient co-twin within 10 days following intravenous Vit. A administration.

1. Palmer, L. S., Ekles, G. H., *J. Biochem.*, 1914, v17, 223.

2. Crook, E. M., El Marsafy, M. K., *ibid.*, 1954, v57, VIII.

3. Dzialoszynski, L. M., Mystowski, E. M., Stewart, C. P., *Biochem. J.*, 1945, v39, 63.

4. Krinsky, N. I., Cornwell, D. G., Oncley, J. L., *Fed. Proc.*, 1956, v15, 113.

5. James, W. H., El Gindi, I. M., *J. Nutr.*, 1953, v57, 97.

6. Kimble, M. S., *J. Lab. Clin. Med.*, 1939, v24, 1055.

7. Ganguly, J., Krinsky, N. I., Mehl, J. W., Deuell, H. J., *Arch. Biochem. Biophysics,* 1952, v38, 275.

8. Diven, R. H., Erwin, E. S., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 601.

9. Church, D. C., MacVicar, R., Bieri, J. G., Baker, F. H., *J. Ani. Sci.*, 1954, v13, 677.

Received November 11, 1958. P.S.E.B.M., 1959, v100.

Effect of Corticotrophin on Guinea Pig Plasma Corticosteroids.* (24632)

HERMAN COHEN AND WILLIAM KLEINBERG
Princeton Labs., Inc., Princeton, N. J.

In view of the similarity between the major blood adrenocortical steroids of man and guinea pig we became interested in using the guinea pig for preclinical assessment of corticotrophins and repository media. Liddle, *et al.*(1) have used the 24-hour urinary steroid output of this species as a method for evaluating effectiveness of corticotrophin. We decided to explore the plasma steroid concentration as possibly offering a more rigorous and rapid evaluation procedure.

Material and methods. Male guinea pigs weighing 350-400 g were used. The animals were sacrificed under light ether anesthesia and exsanguinated from the jugular vein with heparin as the anti-coagulant. Plasma corticoids were determined by the method of Porter and Silber(2). Highly purified corticotrophin (100 USP units/mg) was dissolved in the various menstrua tested *viz.*, 15% gelatin; 4% phosphorylated hesperidin plus 15% gelatin; saline. All injections were given subcutaneously.

Results. Fig. 1 presents distribution of plasma corticoid values of a group of 50 untreated animals. Average value is 43.4 μg/100 ml plasma. These values were further analyzed to determine whether a seasonal variation in plasma steroid concentration existed, but no significant difference was found in values of animals sacrificed in January, June, or September (Fig. 1). The base values are in agreement with those of Done, *et al.* (3) and Boulouard(4) who used a procedure similar to ours for sacrificing their animals. Sobel, *et al.*(5) have also found that the stress produced by ether anesthesia and surgery, such as exposure of the carotid artery

* The technical assistance of Mr. Peter Arnow is gratefully acknowledged. The authors also wish to thank Dr. Herbert Megel for aid in statistical evaluation of the data.