of different sizes (Table 2). These calculations suggest that only particles on the order of the smallest found to date, 10^{-2} μ (*14*), could interact with the geomagnetic field to produce a latitude dependent accretion rate.

If particles greater than 10^{-2} μ in diameter are to impinge upon the earth preferentially at high latitudes through such an interaction, either the particle charge must be greater than we have considered, the velocity less, or both. For purposes of illustration, let us consider a metallic spherule 60 μ in diameter. If the ultimate tensile strength is assumed to be the only factor which limits particle charge, the maximum charge for such a spherule is three orders of magnitude greater than that acquired through minimization of potential energy. It is doubtful, however, that such a high charge could be retained. If particle velocity were $\leq 10^3$ cm sec^{-1}, such a highly charged spherule could be preferentially deposited at a high latitude. Recent indications are that small particles move approximately at escape velocity (*15*), and that slightly lower velocities may characterize particles in orbit about the earth (*16*). However, present knowledge of the processes by which particles are charged in space and of their velocities suggests that the conditions of this illustration are unlikely to be fulfilled. We conclude from these elementary calculations that a simple electromagnetic interaction does not contribute appreciably to the latitude variation in accretion rates as suggested by the data.

In an attempt to explain the apparent variation of particle accretion rates with latitude, it would be useful to conduct new particle collections at a series of surface-based stations along a line of longitude. As the proposed meridian should be determined by the availability of observation sites in contamination-free areas, we recommend consideration of that part of the Pacific Ocean between 150°W and 160°E. There, a sufficient number of islands could be occupied, which, together with drift stations in the Arctic and bases in the Antarctic, could form an adequate network for further observing the latitudinal dependence of particle accretion.

Table 2. Magnetic rigidity of metallic spherules.

Particle diameter (μ)	Magnetic rigidity, $H\rho$ (g cm sec^{-1} emu^{-1})
1	3.0×10^{11}
1	3.9×10^{10}
10^{-2}	3.0×10^{7}
10^{-2}	3.9×10^{6}

RICHARD A. SCHMIDT*
THEODORE J. COHEN
Geophysical and Polar Research Center, University of Wisconsin, Madison

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* Present address: NAS Research Associates, Ames Research Center, Moffett Field, Calif.

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Vitamin K Induced Prothrombin Formation: Antagonism by Actinomycin D

Abstract. *Actinomycin D inhibits vitamin K induced formation of prothrombin in chicks deficient in vitamin K. The administration of actinomycin in doses which inhibit prothrombin formation also inhibits synthesis in the liver of RNA from adenosine triphosphate as detected with adenine-8-C^{14}. The results are consistent with a genetic action of vitamin K in inducing RNA formation for the synthesis of clotting proteins.*

The molecular action of vitamin K has remained an enigma since discovery of the vitamin by Dam in 1935 (*1*), despite a large volume of research directed towards the elucidation of its function. In microorganisms, evidence has been advanced to implicate vitamin K homologs and their chromanols in electron transport and oxidative phosphorylation (*2*). Attempts to explain the antihemorrhagic activity of the K vitamins in mammals on the same basis have not been as successful, although Martius and colleagues suggested in 1954 (*3*) that the defect in prothrombin formation in vitamin K-deficient chicks could be explained on the basis of a primary disorder of energy conservation. Unfortunately for this hypothesis, the uncoupling of oxidative phosphorylation in liver mitochondria from vitamin K-deficient or dicumarol-treated rats and chicks has not been generally confirmed (*4*). Attempts to demonstrate the presence of vitamin K in the prothrombin molecule or effects of vitamin K upon clotting in vitro have likewise failed.

Since administering vitamin K to deficient animals or birds results in the prompt (2 to 6 hours) synthesis and secretion of prothrombin by the liver, vitamin K might directly influence the formation of the messenger RNA required as a template for prothrombin

synthesis. The availability of actinomycin D, a drug which inhibits DNA-dependent RNA synthesis (*5*), made it possible to test this hypothesis.

Day-old white Plymouth Rock chicks were fed a vitamin K-deficient diet (*6*) for 2 weeks. The chicks grew satisfactorily, attaining an average weight of 120 g and demonstrating a marked prolongation of blood-clotting time. In each experiment the deficient chicks were divided into at least three groups (Table 1). One group served as an untreated control. The second received 2 μg of vitamin K_3 (2-methyl-1, 4-naphthoquinone) per 100 g of body weight, the dose being given in sesame oil by tube into the crop. The solution administered contained 2 μg per milliliter. The third group received intraperitoneal doses of actinomycin D, in propylene glycol, ranging from 40 to 800 μg per 100 g of body weight in volumes of 0.2 to 0.5 ml 2 hours prior to the administration of vitamin K_3. Blood was drawn by heart puncture for measurement of prothrombin time by the method of Quick and Grossman (*7*) 6 hours after the administration of vitamin K.

In order to evaluate the effect of actinomycin upon the synthesis of hepatic RNA in treated birds, 5 μc of adenine-8-C^{14} (9.3 mc/mmole) were administered twice to chicks from selected groups, 6 hours and 4 hours prior to sacrifice. The liver was quickly removed, chilled, and homogenized with cold 0.5*M* perchloric acid; the homogenate was centrifuged at 800*g* for 30 minutes. Adenosine-5′-phosphate was isolated from the supernatant by the method of Edmonds and LePage (*8*). The RNA in the washed residue was differentially hydrolyzed by the method of Schmidt and Thannhauser (*9*) and the resulting adenosine-2′- and adenosine-3′-phosphates were isolated on Dowex-1 formate. The adenine nucleotides were dissolved in Hyamine and counted in diphenyloxazoletoluene in a Packard scintillation spectrometer.

The effect of actinomycin in preventing the appearance of prothrombin in the blood of vitamin K-deficient chicks given vitamin K_3 is shown in Table 1. The deficient chicks uniformly showed prothrombin times in excess of 240 seconds. When 2 μg of vitamin K_3 were given by mouth, the prothrombin time decreased to 75 $\pm$ 27 (S.D.) seconds in 6 hours.

Table 1. Effect of vitamin K and actinomycin D upon the prothrombin time of vitamin K-deficient chicks.

Chicks (No.)†	Actinomycin (μg/100 g)	Vitamin K_3 (μg/100 g)	Number of chicks with prothrombin, times* indicated (sec)				
			30–70	71–100	101–160	161–240	>240
			Experiment No. 1				
6(1)	40	0	0	0	0	0	6
10	0	2	0	2	6	2	0
6(1)	40	2	1	0	3	1	1
			Experiment No. 2				
10	0	0	0	0	0	0	10
10	0	2	2	6	2	0	0
6	200	2	0	0	3	1	2
6	400	2	0	0	1	3	2
2(4)	800	2	0	0	0	0	2
			Experiment No. 3				
8	0	0	0	0	0	0	8
10	0	2	2	4	4	0	0
10(1)	800	2	0	3	1	2	4
			Experiment No. 4				
9	0	0	0	0	0	0	9
9	0	2	1	6	2	0	0
5(5)	800	2	0	0	0	1	4
			Experiment No. 5				
9	0	0	0	0	0	0	9
9	0	2	1	2	4	2	0
11(5)	800	2	0	0	1	2	8

* Prothrombin times for normal human subjects ranged from 18 to 20 seconds; for normal chicks, from 20 to 30 seconds. A normal control sample was run with each batch of chick plasma. † The number of chicks listed corresponds to the number of observations made. The numbers in parentheses denote the number of chicks dying during the experimental period of 6 hours, which were consequently not studied.

In the presence of a prior dose of actinomycin, this induced prothrombin formation was greatly retarded. A slight effect was noted at a dosage of 200 μg per 100 g of body weight, which increased to a marked effect when the dosage was increased fourfold (800 μg/100 g). At this high dosage, 83 percent of all chicks given actinomycin and then vitamin K_3 showed prothrombin times in excess of 160 seconds. With vitamin K_3 alone, only 8 percent showed such prolonged prothrombin times. In other experiments, actinomycin at 800 μg per 100 g of body weight had no effect on the normal prothrombin times of chicks pretreated with vitamin K_3 for several days prior to the injection of actinomycin.

Table 2 shows that adenine-8-C^{14} was well incorporated into liver 5′-AMP (*10*) in both untreated and actinomycin-treated chicks, showing that the formation of adenine nucleotide was unaffected by the drug. Since 5′-AMP is in isotope equilibrium with ADP and ATP in living cells, the specific activity recorded can be regarded as similar to that of the triphosphate precursor of RNA-bound adenine nucleotide. The agreement between the specific activities of the 2′- and 3′-AMP-RNA hydrolysis products provides good evidence for their origin in RNA. The ratio of specific activities

Table 2. Effect of actinomycin on formation of 5′-AMP and RNA in livers of seven chicks.

Specific radioactivity of products (per μmole)*			Ratio specific activities $\frac{\text{2′- and 3′-AMP}}{\text{5′-AMP}} \times 10$
5′-AMP	2′-AMP	3′-AMP	
	No actinomycin		
92,200	19,900	18,600	2.08
120,000	16,200	11,800	1.17
61,200	12,100	12,100	1.98
	160 μg of actinomycin per 100 g		
46,700	4,450	5,310	1.07
59,200	7,800	8,200	1.35
	800 μg of actinomycin per 100 g		
67,600	2,570	2,680	0.39
44,400	1,580	2,590	0.45

* Specific radioactivity is expressed in terms of disintegrations per minute (DPM) per micromole (2.2×10^6 DPM = 1 μc).

of product to precursor, shown in the last column of Table 2, is thus a measure of relative incorporation rate of ATP into liver RNA. Thus, at a dosage of 160 μg of actinomycin per 100 g of body weight, the change in incorporation rate was negligible. At doses that inhibit prothrombin synthesis (800 μg/100 g), the synthesis of RNA from ATP was inhibited about 80 percent.

These data suggest that vitamin K acts to induce prothrombin synthesis. Based on the model proposed by Jacob and Monod (*11*) the following hypothesis is advanced. In the absence of vitamin K a regulatory gene represses the activity of the operon concerned with the elaboration of the vitamin K-dependent clotting proteins, namely prothrombin, plasma thromboplastin antecedent, plasma thromboplastin component, and proconvertin. Vitamin K may then act to derepress the operator by combining with the repressor molecule. The structural gene components of the operon are thus freed to elaborate their respective messenger RNA's which result in the synthesis of their respective proteins. The effect of actinomycin D in blocking the action of vitamin K in these experiments is consistent with the known action of actinomycin in blocking DNA-dependent RNA synthesis (*5*). This hypothesis also explains the failure of vitamin K to act in hereditary hypothrombinemic states (*12*) in which it may be assumed that the structural gene components of the operon are defective.

This work suggests the possibility that all of the fat-soluble vitamins operate to control the synthesis of specific proteins and enzymes in the highly differentiated mammal. This speculation is strengthened by the parallel similarity of the other fat-soluble vitamin deficiency diseases to genetically conditioned disorders. Evidence already exists that some of the chemically related steroid hormones act at the genetic level (*13*).

ROBERT E. OLSON

Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, Pittsburgh 13, Pennsylvania

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Dependence of a Gm(b) Antigen on the Quaternary Structure of Human Gamma Globulin

Abstract. *The antigens associated with the Gm(b) factor of human 7S γ-globulin differ in Caucasoids and Negroids. The studies reported here show that an antigen, Gm(b^w), associated with the Gm(b) of whites but not with that of Negroes, is present on the S (slow) fragment of 7S γ-globulin obtained by digestion with papain or pepsin. All other Gm antigens thus far studied have been found on the F (fast) fragment. The antigens Inv(a) and Inv(b) were detected on isolated L-chains, and Gm(a) and Gm(b) were detected on isolated H-chains, but Gm(b^w) was detected on neither. Recombining the H- and L-chains restored activity for Gm(b^w) comparable to that of intact γ-globulin.*

The hereditary antigens of human 7*S* γ-globulin are determined by codominant alleles at two different loci, Gm and Inv (*1*). Studies on the distribution of Gm and Inv antigens on fragments of 7*S* γ-globulin produced by papain digestion have shown that the Gm(a) and Gm(b) antigens are located on the F (fast) fragment, while the Inv(a) and Inv(b) antigens are located on the S (slow) fragment (see *2*).

Steinberg and Wilson (*3*) have shown that Gm(b+) Caucasians possess an antigen, termed Gm(b^w), which is absent in Gm(b+) Negroes. This paper is a report of our first studies of the distribution of the Gm(b^w) antigen on enzymatically produced fragments of human 7*S* γ-globulin.

Gamma-globulin (7*S*) was isolated from the serums of four Gm(b^w+) donors by elution from diethylaminoethyl (DEAE) cellulose according to the method of Sober and Peterson (*4*). The γ-globulin was digested with papain in the presence of cysteine according to the method of Porter (*5*). The F and S fragments obtained were separated by chromatography on DEAE-cellulose. The S fragment preparations were further purified by gel filtration with Sephadex G-100. Immunoelectrophoresis and double diffusion in agar (Ouchterlony technique) failed to reveal contaminating material in the F fragment preparations. Some contaminating, undigested 7*S* γ-globulin was detected in the S fragment preparations by the Ouchterlony technique, but not by immunoelectrophoresis.

As already noted, the Gm(a) and Gm(b) antigens are located on the F fragment, while the Inv(a) and Inv(b) antigens are on the S fragment (*2*). We have confirmed these observations for the antigens detected by the standard reagents, but we have consistently found the Gm(b^w) antigen on the S fragment. This is illustrated in Table 1 by data obtained from one of the donors.

Similar results have been obtained when 7*S* γ-globulin was digested with pepsin according to the method of Nisonoff *et al.* (*6*). This method produces an S fragment similar to that produced by papain digestion, but no F fragment is recovered (*6, 7*). Samples of purified pooled 7*S* γ-globulin and 7*S* γ-globulin isolated from two Gm(b^w+) donors were digested with pepsin for 18 to 48 hours. Traces of undigested γ-globulin were detected by the Ouchterlony technique in samples digested for 18 hours, but not in samples digested for 48 hours. After 48 hours of digestion of 7*S* γ-globulin from a Gm(a—b+b^w+), Inv(a—b+) donor, Gm(b) was not detectable at a protein concentration of 8.8 mg/ml. On the other hand, Gm(b^w) was detected at a protein concentration of 0.05 mg/ml, as compared to 0.10 mg/ml for intact 7*S* γ-globulin, and Inv(b) was detected at 0.03 mg/ml as compared to 0.009 mg/ml for intact γ-globulin (*8*) (Table 2).