TABLE II. Thromboplastin from Normal Rabbit Brain Compared to That from Dicumarolized Rabbit Brain. Procedure as in Table I, using dicumarol rabbit plasma as clotting reagent. Plasma from same rabbit as dicumarol serum used in Table I.

Material tested	Clotting time, sec. Normal thromboplastin	Clotting time, sec. Dicumarol thromboplastin
Saline sol.	64	190
Normal rabbit serum 1-100	15	15
Dicumarol rabbit serum 1-100	50	

treatment with dilute chicken serum but not by rabbit serum (Table IC). In order to be effective, the serum must be homologous to the thromboplastin, not to the plasma.

Thromboplastin from the brain of a dicumarolized rabbit has been reported to be relatively inactive with respect to dicumarol plasma(7-9). Pretreatment with dilute normal homologous serum removed this difference and greatly shortened the prothrombin time of dicumarol plasma (Table II). These data are included for the purpose of comparing the effects obtained with normal heterologous and with dicumarol homologous plasmas.

Summary. Species specificity of thromboplastin is largely eliminated by brief treatment of the thromboplastin with dilute homologous serum. This appears to be an example of the cothromboplastin reaction.

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Ineffectiveness of Parenteral Pyroxidine in Relieving or Preventing the Egg White Syndrome in the Rat.* (19259)

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The report of MacKay and Barnes(1) that signs of the egg white syndrome were cured in the rat by the injection of vit. B_6 when corn oil was included in the ration, has been interpreted by Sherman(2) in a recent review, to offer evidence that ". . . pyridoxine and essential fatty acids act mutually to enhance or replace biotin."

Salmon and Goodman(3) observed that the severity of the skin lesions in the egg white syndrome were decreased and their character altered somewhat by the presence of linseed oil, butter fat or hydrogenated cottonseed oil in the diet. They attribued this effect to the unsaturated fatty acids which were supplied by the fats. On the other hand, Nielson and Elvehjem(4,5) found that neither corn oil (5 or 10%) nor pyridoxine (100 to 125 μg orally, daily) offered protection against spectacle eye condition or paralysis in their rats on egg white rations. However, as there was the possibility that the effectiveness of pyridoxine in the study of MacKay and Barnes might have been attributable to their use of the parenteral method of administering the vitamin, this was explored.

Experimental. The basal ration had the following percentage composition: egg white

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TABLE I. Effect of Parenteral Administration of Pyridoxine and of Biotin on Manifestations of Egg White Syndrome in the Rat.

Group	No. of rats	Type of exp.	Vitamin inj. mg B_6	Vitamin inj. μg biotin	Severity of symptoms	Days on dose	% of groups C and D showing initial signs of deficiency at ..... days: 17	19	22	27
A		Curative	.2							
	4				3+	0				
	4				3+	14				
	3				3+	21				
	1				4+*	58				
B		"		1						
	8				4+	0				
	8				2+†	14				
	8				0 to 1+†	21				
C		Preventive	.2							
	15				2+		47	47	60	93
D		Control								
	19				2+		33	55	77	100

* Symptoms of the 58th day included: pronounced "spectacle eyes," swollen red bare lips, extensive alopecia and dermatitis, and severe "paralysis."

† Initial symptoms were measurably modified in 1 wk, were inconspicuous after 2 wk of therapy, and at the end of 3 wk there remained only a slight bareness around the eyes of 2 rats and a bristly appearance of the new thick hair.

20, sucrose 71, salt mixture(6) 4, and corn oil 5. The following weights of vitamins were incorporated per each 100 g of ration: 0.2 mg thiamine, 0.2 mg riboflavin, 0.4 mg pyridoxine, 2.0 mg pantothenic acid, and 0.1 g choline. Two drops of a cod liver-corn oil mixture of the following composition were given each rat every 5 days: 45 ml corn oil, 50 ml cod liver oil, 5 g alpha-tocopherol, and 0.4 g methyl napthoquinone.

Moderately pronounced signs of the egg white syndrome were produced in male albino rats in the short period of 35 days, thus giving no evidence that the corn oil of the ration delayed the onset of the symptoms significantly in comparison with earlier experiments in which corn oil was not fed. From the 36th day Group A (Table I) received daily injections of 0.2 mg of pyridoxine hydrochloride. At the end of 14 days, a period which had sufficed for almost complete cure in the rats of MacKay and Barnes, the condition of these animals was fully as pronounced as before; this was also true for the rats which were injected beyond this period (Table I).

Three rats in Group A received, from the 14th or the 21st day, an additional daily supplement namely, a dose of biotin by stomach tube, in an attempt to determine whether or not the injections of pyridoxine would make a measurable difference in the use of biotin by the animals. However, the response (not recorded in Table I) proved to be similar to that obtained in the control rats from similar doses of biotin alone, administered by stomach tube.

In contrast to the lack of benefit obtained from injections of pyridoxine, the curative effect of parenterally administered biotin may be seen in Table I, Group B. To exaggerate the contrast, the symptoms were allowed to become more severe in Group B than in Group A before injections were begun. One μg biotin per day was injected for 21 days, resulting in a rapid regression of the symptoms (Table I). In a more crucial test than the "curative" one above, 200 μg doses of pyridoxine hydrochloride were injected daily into 15 weanling rats from the time that they were first put onto the egg white ration. The record of Groups C and D in Table I shows that there was no appreciable difference between these injected rats and their controls without injections in regard to the time of onset of signs of biotin deficiency. Hence, parenteral doses of pyridoxine failed to enhance or replace biotin for the rat under the conditions of this

experiment in which essential fatty acids were also available.

In attempting to account for the disparity in the results in the two laboratories, one might conjecture that the deficiency symptoms produced by MacKay and Barnes were primarily those of pyridoxine lack. No evidence was presented by them that the symptoms would respond to the administration of biotin. The method of producing the manifestations over so prolonged a period would further leave their interpretation open to question. It has been the experience of several laboratories(7,8) that the sex and initial weight of the rats placed on egg white rations are very important factors in the development of the characteristic egg white syndrome. Male rats show earlier and more marked manifestations than females. Gradations in depletion may also be seen between groups started at 35, 45 and 55 g initial weight respectively; the larger the animals, the more resistant they are to the onset of the characteristic signs of depletion. Hence, it may be significant that the rats of MacKay and Barnes were 40-day-old females with an initial body weight of about 70 g, whereas the rats in the present study were 21-day-old males with an average body weight of 37 g. Inasmuch as the source of pyridoxine in the basal ration of MacKay and Barnes was not the pure vitamin but 5% of yeast, the degree of its availability was not established. The exact requirement would also be conditioned by the amino acids furnished by the basal ration.

Summary. Parenteral doses of pyridoxine failed to cure or to delay the onset of the egg white syndrome in rats on a ration which included the essential fatty acids. The assumption by others, therefore, that ". . . pyridoxine and essential fatty acids act mutually to enhance or replace biotin," is not supported by these experiments with the rat.

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Adrenocorticotrophic Hormone: Stability Studies.* (19260)

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The effects of hydrogen ion concentration, ionic strength and temperature upon the biological activity of adrenocorticotrophic hormone (ACTH) have been investigated in order to define conditions which may be expected to produce little or no inactivation of the hormone. Stability data are obviously of considerable practical importance for the successful isolation of the hormone from pituitary, for the evaluation of its potency by bioassay, and for the preparation of solutions suitable for clinical use. In addition, conditions which inactivate pituitary contaminants and which have little or no effect upon ACTH activity can be applied to the biological puri-

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