individual or of a patient with disease. The appearance and length of the β spike depends somewhat on the dietary state of an individual, the amount of fat present in the blood, and the time of withdrawal of the blood sample. It is interesting to note that in the classical electrophoretic patterns reported by Longsworth and coworkers the β spike was evident in most patterns and was long and narrow(4). On the other hand, patterns reproduced in more recent publications very often show a very short β spike or in some cases the complete absence of this spike. It is also interesting to observe that in the present investigation plasma and serum samples from normal children exhibit shorter β spikes than those from normal adults.

Before drawing any definite conclusions as to the cause of the slight differences between the length of the β spike in patterns from normal individuals or those from patients with disease, the origin of the β spike must be determined. We plan to extend this investigation to include a study of the origin of the β spike in electrophoretic patterns of plasma and serum.

Summary. 1. Electrophoretic patterns of approximately 1100 plasma and 500 serum samples have been examined for changes in the length of the β spike. These samples were obtained from normal children and adults and from patients with poliomyelitis, diabetes, rheumatic fever, arthritis, and liver disease. 2. Minor changes occurred in the average length of the β spike in electrophoretic patterns from plasma and serum when patients with disease were compared with normal individuals. These changes were also observed when patterns from patients with one disease were compared to patterns from other diseases. These changes were not marked enough to differentiate between diseases or between normal individuals and those with disease.

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Effect of Lyxoflavin on Growth of Baby Pigs Fed a Synthetic Diet. (19469)

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In 1949 Pallares and Garza(1) isolated from human heart myocardium a pentoseflavin which they identified as l-lyxoflavin. The significance of the presence of lyxoflavin in human heart muscle was not discussed by these investigators. However, Emerson and Folkers(2) have suggested the possibility that lyxoflavin may be a new member of the vit. B complex. Lyxoflavin differs from riboflavin only in the configuration of the groups about carbon 4 of the pentose side chain.

The present experiments were designed to study the effect on growth of feeding lyxoflavin to baby pigs on a "synthetic milk" ration containing all of the known nutrients.

Experimental. Baby pigs from one to 2 days of age were used as experimental animals. The technic of feeding and care of the animals has been described by Johnson *et al.*(3). The composition of the basal rations used in these experiments is given in Table I. In addition

TABLE I. Composition of Basal Diets.

	Casein diet, %	Alpha-protein diet, %
Casein (vit.-free)	25	—
Alpha-protein	—	24.6
DL-Methionine	—	.4
Sucrose	68	68
Cottonseed oil	1	1
Minerals	6	6

The above materials were made into a "milk" containing 19.5% solids.

Vitamins* added/liter "synthetic milk"

	mg
Thiamine hydrochloride	1
Riboflavin	2
Pyridoxine hydrochloride	2
Ca pantothenate	12
Nicotinic acid	4
Inositol	40
Choline	400
Biotin	.016
Ascorbic acid	.016
Folic acid	.08
2-Methyl-1,4-napthoquinone	.4
Alpha tocopherol acetate	1.54
Vit. A	3000 I.U.
D_2	300 I.U.
B_{12}	.8 μg/kg body wt /day, given by inj.

* Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, Ca pantothenate, nicotinic acid, biotin, ascorbic acid, crystalline vit. B_{12}, alpha tocopherol acetate, choline chloride and synthetic L-lyxoflavin were supplied by Merck and Company, Inc., Rahway, N. J., through the courtesy of Dr. D. F. Green and Dr. Karl Folkers. Folic acid was supplied by Lederle Laboratories, Inc., Pearl River, N. Y., through the courtesy of Dr. T. H. Jukes.

the pigs were given protamone at a level of 0.01% of the dry matter of the ration in order to enhance the development of a deficiency state by increasing the metabolism of the animal. In a preliminary experiment it was found necessary to substitute "milk" containing 30% of lard for the low fat milk during the first few days. The amount was gradually decreased so that by the end of the first week the pigs were receiving the "synthetic milk" as described in Table I. In a preliminary experiment protamone was fed at a level of 0.1% of the dry matter of the diet. This level of protamone resulted in a high mortality rate and the level was therefore reduced in the subsequent experiments.

A summary of the total performance of the pigs in Exp. 1 and 2 is shown in Tables II and III. In Exp. 1 the pigs received the casein basal ration for 49 days. Group 2, which received lyxoflavin supplementation (4 μg per g of dry matter), outgained the control group at a highly significant rate on significantly less dry matter consumed per kg gain in body weight. Because of the loss of several pigs in this experiment due to the physical nature of the diet, alpha-protein was substituted for the casein in Exp. 2. The data of this experiment are given in Table III. Using the analysis of covariance (Fisher(4), and Snedecor(5)), the difference in weight gains brought about by the addition of 4 μg of lyxoflavin per g of dry matter consumed was found to be very highly significant ($P < 0.001$).

To study the presence of lyxoflavin in urine or tissues a differential assay procedure was developed by assaying standard riboflavin and standard lyxoflavin solutions and mixtures of the 2 both microbiologically and fluorometrically. For the fluorometric assay the solutions were adsorbed on florisil and the riboflavin and/or lyxoflavin was eluted by passing 20 to 23 ml of a solution of 20% pyridine in 2% acetic acid through the column. The eluate was made up to 25 ml. To an aliquot of the eluate was added 2 drops of glacial acetic acid and 2 drops of 4% potassium permanganate, and the solution was swirled and left to stand for 2 minutes. The solution was then cleared with 3 to 4 drops of 3% hydrogen peroxide and the fluorescence determined in a Coleman 12A fluorophotometer. A dilute solution of sodium fluorescein was used as primary standard. The results of a series of fluorometric

TABLE II. Response of Baby Pigs to Lyxoflavin (Exp. 1).

Items compared	Group 1 basal	Group 2 basal + lyxoflavin
No. of pigs	3	4
Avg initial wt, kg	1.91	1.82
" final " "	18.25	19.96
" total gain "	16.34	18.14*
" daily food consumed, kg	.52	.53
" food consumed (per kg gain)	1.56	1.43†

* Highly significant over Group 1 ($P = <.01$).
† Significant over Group 1 ($P = <.05$).

TABLE III. Response of Baby Pigs to Lyxoflavin (Exp. 2).

	Group 1 basal	Group 2 basal + lyxoflavin
No. of pigs	4	5
Avg initial wt, kg	1.46	1.41
" total gains (8 wk)	14.73	16.40*
" food consumed (per kg gain)	1.72	1.64

* Significant over Group 1 ($P = <.001$).

TABLE IV. Lyxoflavin and Riboflavin Excretion in Urine of Pigs (mg per Day).

Group No.	No. of pigs	Lyxoflavin intake	Riboflavin excreted*	Riboflavin +lyxoflavin excreted†	Lyxo-flavin excreted‡	% lyxoflavin intake excreted in urine
I Exp. 1	2		2.3	2.4		
2	1		2.5	2.6		
Avg both exp.			2.4	2.5		
II Exp. 1	3	2	2.3	3.1	1.2	60
2	5	2.7	2.75	3.9	1.8	66
Avg both exp.		2.4	2.57	3.6	1.58	65.8

* By *L. casei* assay. † By fluorescence assay.
‡ Difference between casei assay and fluorescence assay multiplied by 100/58.7 and corrected for the 106 (avg) μg per day higher riboflavin excretion by fluorometric assay of the Group I pigs.

readings gave values for the lyxoflavin of 55.5 to 60.4% (average 58.7%) of the riboflavin reading at that same concentration.

A microbiological assay on these standard solutions was carried out simultaneously using *Lactobacillus casei* as the test organism. From the results of this assay it was apparent that lyxoflavin did not replace riboflavin in the growth of this organism.

The lyxoflavin content of the unknowns may then be calculated as follows:

$$\frac{(\text{Fluorometric assay value}) - (\text{microbiological assay value}) \times 100}{58.7}$$

In order to determine whether riboflavin may be converted into lyxoflavin in certain tissues of the body, homogenates of liver and heart were incubated in phosphate buffer in the presence of 50 μg of added riboflavin per sample (1-2 g) of tissue. The solutions were incubated at 37° C under toluene for 4, 8, 16, and 24 hour periods. At the conclusion of the incubation periods, the riboflavin content was determined both fluorometrically by the method of Hodson and Norris(6) and microbiologically by the *Lactobacillus casei* method of Snell and Strong (as given by Johnson (7)). No change in riboflavin content and no evidence of lyxoflavin formation was found following incubation.

Limited data on the lyxoflavin content of heart muscle indicate the possibility of some lyxoflavin being stored in this organ. Using the average values obtained it was found that the hearts from the lyxoflavin-fed pigs averaged 0.60 μg per g higher in riboflavin content by fluorometric assay than by microbiological assay. On the other hand the heart tissue of the pigs which did not receive lyxoflavin averaged only 0.175 μg per g higher in riboflavin content by fluorometric assay than by microbiological assay. If one assumes this 0.175 μg to be due to fluorescent substances other than lyxoflavin there is still 0.425 μg per g unaccounted for in the heart tissue of lyxoflavin-fed pigs which may be due to lyxoflavin. Since it was found that lyxoflavin will fluoresce only 58.7% as much as riboflavin, one may calculate a lyxoflavin content of approximately 0.7 μg per g in the heart tissue.

Twenty-four hour urines were collected in both experiments. The urine was analyzed for lyxoflavin using the same differential assay. Paper strip chromatograms were also run on the urine. The data on urinary excretion of lyxoflavin are presented in Table IV. An average 68.1% of the lyxoflavin intake was accounted for in the urine by this procedure after correcting for interfering fluorescent substances. Sixteen different solvent systems were used in an attempt to separate riboflavin and lyxoflavin on a paper strip chromatogram. None of the solvent systems tried separated these two closely related compounds. However, paper strip chromatograms of the urine of pigs fed lyxoflavin, using a solvent system of butanol, pyridine, and water, showed a violet spot which was not present in the urine of pigs fed the basal ration only. This metabolite differed from both lyxoflavin and riboflavin.

Discussion. Emerson and Folkers(8) have

reported an increased rate of gain in rats fed lyxoflavin as compared to the basal group. From the results of the experiments reported herein, it appears that lyxoflavin also has growth promoting activity in baby pigs when the basal ration contains a minimum amount of fat and contains protamone to enhance the deficiency state.

It appears possible that lyxoflavin may be a new member of the vit. B complex with a biological role different from that of riboflavin and other known vitamins. However, the fact that the baby pigs do quite well on the basal ration which is devoid of any known lyxoflavin might indicate that lyxoflavin exerts its effect in a manner similar to that of the antibiotics or of the surfactants(9) or other growth stimulants, rather than due to the vitamin nature of the compound. The high amount of the lyxoflavin fed which was excreted in the urine might indicate tissue saturation and implies that a lower level would have been as effective as the level used. In the case of riboflavin approximately 30% of an intake of 11 μg per g was excreted in the urine as compared to 68.1% of 4 μg per g for lyxoflavin.

Lyxoflavin is apparently not converted into riboflavin in the animal body as Emerson and Folkers(8) report that synthetic lyxoflavin is devoid of riboflavin activity in rats by the standard assay. We have confirmed this by *in vitro* studies and have also confirmed their report that lyxoflavin is inactive for *Lactobacillus casei*.

It appears that some lyxoflavin may be stored in the heart, however, the amount is small and with a differential assay it is more difficult to determine these minute quantities than with a direct assay.

Summary. 1. The addition of 4 μg of synthetic l-lyxoflavin per g of dry matter consumed in 3 experiments significantly increased the rate of gain and efficiency of feed utilization of baby pigs fed a low fat basal ration including 0.01% protamone. 2. Using a differential assay procedure for lyxoflavin it was found that approximately 67% of the lyxoflavin intake was excreted in the urine. 3. *In vitro* studies of heart and liver tissue did not indicate any conversion of riboflavin into lyxoflavin.

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Shwartzman Phenomenon II. Suppressive Action of HN_2 on Antigen-Antibody Provocation. (19470)

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The basic experiment demonstrating the phenomenon of local tissue reactivity (Shwartzman phenomenon) consists of the intradermal injection of a small amount of a suitable bacterial filtrate (preparatory injection), followed after some 24 hours by an intravenous injection of a quantity of the same material (provocative injection). In susceptible rabbits, a zone of hemorrhagic necrosis appears at the site of the preparatory injection 3 to 5 hours after the intravenous injection of filtrate(1). The phenomenon can