terpret the apparent protective effect obtained by Wood and Rusoff[3] as being due to a well recognized and non-specific peritoneal protection afforded by certain inert substances when injected previous to the inoculation of infectious agents by the same route. Our results suggest that these two neurotropic viruses do not enter the central nervous system directly by way of the vascular channels as is the case with such an agent as cocaine, the passage of which into the central nervous system tissue may be altered by trypan red.

We wish to acknowledge the technical assistance of Doctor David Zealear and Miss Sylvia Bowditch in one phase of this study.

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Unidentified Growth Factor(s) Needed for Optimum Growth of Newborn Pigs.

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An unidentified growth factor(s) (the animal-protein factor, factor X,[1] vitamin B_{12},[2] vitamin B_{13},[3] etc.) has been reported to be present in certain concentrates such as anti-pernicious anemia liver extract. It has been reported that this factor(s) will improve the growth rate of the rat,[4] the dog,[5] the fox,[6] and the chick.[7]

Since it has been shown[8,9] that newborn pigs can be raised successfully to weaning age on a synthetic ration made up to simulate milk, this appeared to be a suitable technic for determining whether the pig requires such an identified growth factor(s). Carey *et al.*[1] have reported that casein supplies significant amounts of factor X to the rat, and for this reason the "vitamin-free" casein used in previous studies[8,9] was replaced in the first experiment by an isolated soybean protein (alpha-protein).* Methionine was added to the diet, since this alpha-protein is deficient in sulfur amino acids.[10] The composition of the rations fed is given in Table I.

Experimental. Experiment I. Seven 2-day-old Duroc Jersey-Chester White cross-bred pigs from the same litter were divided into 3 groups on the basis of weight and age. The pigs were housed in individual raised wire cages in a heated building and were fed the following diets *ad libitum*. Group 1 received the casein "synthetic milk" ration[9] (Diet A, Table I); Group 2 received the alpha-protein "synthetic milk" (Diet B); and Group 3 received Diet B plus Reticulogen,† a 20-unit/ml anti-pernicious anemia liver extract. Individual feed consumption records were

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* The alpha-protein was generously supplied by the Glidden Co., Chicago, Ill., through the courtesy of Dr. J. L. Gabby.

† The reticulogen (20-unit anti-pernicious anemia liver extract) was generously supplied by Eli Lilly and Co., Indianapolis, Ind., through the courtesy of Dr. E. C. Kleiderer.

TABLE I.
Composition of "Synthetic Milk" Rations.

	Diet A Casein diet (Group 1)	Diet B Alpha protein diet (Group 2 and 3)	
Casein (Labco, vitamin-free)	30		
Alpha protein (isolated soybean protein)	—	29.7	
Methionine*	—	0.3	
Glucose (cerelose)	37.4	37.4	
Mineral salts†	6.0	6.0	
Lard	26.6	26.6	
Reticulogen	—	0.33	ml/pig/day Group 3, Expt. I

Made up and homogenized into a milk containing 13% solids including 4% lard (liquid basis).

The following vitamins‡ were added per 1000 g of milk:

Thiamine	0.65 mg	*p*-aminobenzoic acid	2.6 mg
Riboflavin	1.30 "	Pteroylglutamic acid	0.052 "
Pyridoxine	1.30 "	Biotin	0.01 "
Calcium pantothenate	7.8 "	α-tocopherol acetate	1.0 "
Inositol	26.0 "	2-methyl-1,4 naphthoquinone	0.26 "
Choline	260.0 "	Vit. A	1000 I.U.
Nicotinic acid	2.67 "	Vit. D	100 "

* The *dl*-methionine was furnished by E. I. DuPont de Nemours and Company, Inc., New Brunswick, N.J.

† See Johnson *et al.*[9]

‡ The thiamine chloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid, biotin, and α-tocopherol acetate used in this experiment were generously supplied by Hoffman-La Roche, Inc., Nutley, N. J. Pteroylglutamic acid was supplied by the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y. Inositol was donated by A. E. Staley Manufacturing Co., Decatur, Ill. Hyflavin (a highly water-soluble riboflavin) was furnished by Endo Products, Inc., New York City.

TABLE II.
Results of Experiment I.

Items compared	Groups and treatments I Casein milk	II Isolated soybean protein milk plus DL-methionine	III Same as II plus reticulogen
No. of pigs started	2	2	3
Av initial wt, g	1780	1785	1523
" wt at 21 days, g	6940	6125	5600
" final wt, g	14610	13530	19960
" daily gain during test, g	231.5	209.8	326.9
" " " to 21 days, g	245.2	206.6	195.2
" " " 21 to 56 days, g	222.5	211.6	404.1

kept and the pigs were weighed at weekly intervals.

Reticulogen was given to the pigs in Group 3 at the rate of 0.33 ml per pig daily. To reduce intestinal synthesis of unidentified growth factors, sulfathaladine‡ was added to the diet at the rate of 2 g per liter of milk. The fat-soluble vitamins were added to the milk during homogenization, and the water-soluble vitamins were added to the milk at the time of feeding.

The gains as ratios of attained weight to initial weight for the 3 groups are plotted in Fig. 1, and the data are summarized in Table II. At 8 weeks the pigs in Group 3 (Reticulogen) had gained significantly more than those in Group 2 ($P = 0.007$)[11] or those in Groups 1 and 2 ($P < 0.001$).[11]

The photographs in Fig. 2 illustrate the

‡ Sulfathalidine (phthalylsulfathiazole) was generously supplied by Sharp and Dohme, Philadelphia, Pa., through the courtesy of Dr. S. F. Sheidy.

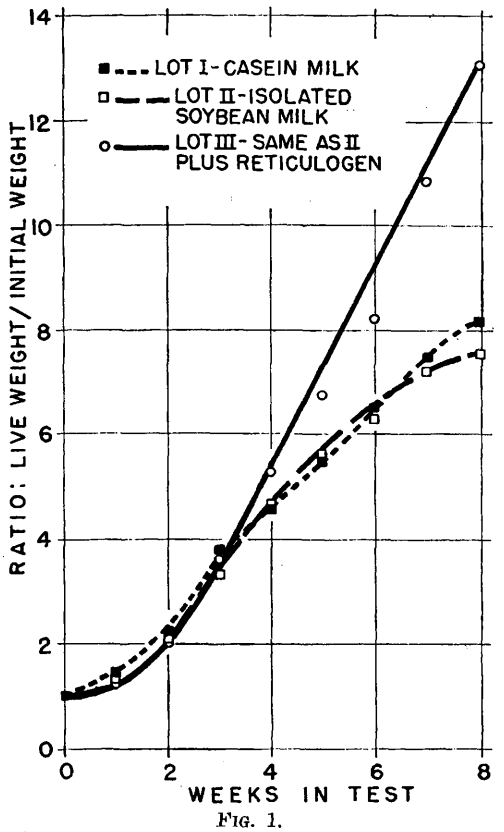

FIG. 1.
Average gains expressed as ratio of weight to initial weight.

improvement in thrift, size, condition and amount of haircoat, and soundness of feet and legs when Reticulogen was given as a source of the unidentified growth factor(s).

Normal hemoglobin levels and normal red and white cell counts were found in all pigs at 8 weeks.§

At the close of the experiment all pigs were placed together and given access to green pasture and a good farm ration for weanling pigs. After 3 weeks there had been practically no gain in the pigs from Groups 1 and 2, while those from Group 3 continued to gain normally. Only after 5 weeks on a good farm ration did the pigs on the unsupplemented rations overcome their growth factor(s) deficiency.

Experiment 2. In order to confirm the results of Experiment 1 in which only a small number of animals were used, a second experiment was carried out in which 21 two-day-old Duroc-Jersey pigs were used. In this experiment vitamin-free casein was used as the protein source in the ration, since the results of Experiment 1 had indicated that this vitamin-free casein was almost as deficient in the unknown factors present in Reticulogen as was alpha-protein.

The basal ration (Diet A, Table I) was fed to a group of 9 baby pigs. Another group of 12 baby pigs received the same ration plus 0.25 ml of Reticulogen per pig daily. The pigs were housed and fed as in Experiment 1.

Again, after the 8-week experimental period, the animals in the group receiving Reticulogen had gained significantly more than those on the basal ration ($P = <0.001$). These results are summarized in Table III.

11 Fisher, R. A., Statistical Methods for Research Workers, 9th Ed., Oliver and Boyd, Ltd., London, 1944.

§ We are indebted to Dr. Marian F. James for these determinations.

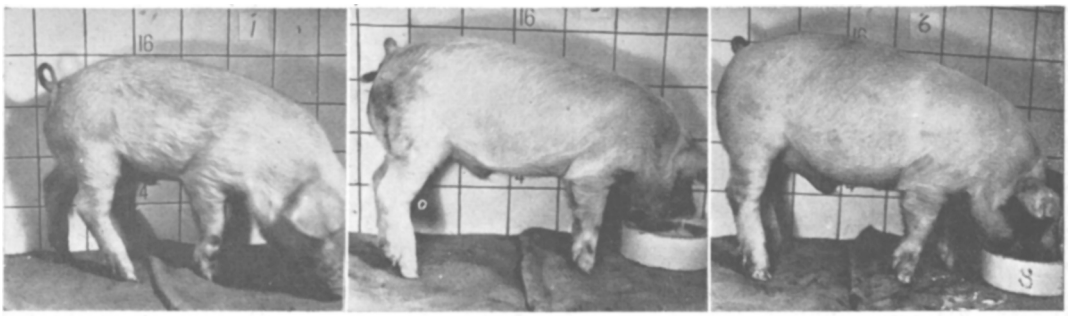

FIG. 2.
Representative pigs from the 3 lots. Left, Pig. No. 1 from the casein lot. Middle, Pig No. 3, from the alpha-protein lot. Right, Pig No. 6, from the alpha-protein + Reticulogen lot.

TABLE III.
Weight Gains in Experiment II.

	Group	
	I Basal ration A	II Basal ration A + reticulogen
No. animals	9	12
Av initial wt	1.56 kg	1.60 kg
Av final wt at 8 wks	16.0 "	21.6 "
Av gain in wt for 8 wks	14.4 "	20.0 "

Summary. The addition of Reticulogen (a 20-unit anti-pernicious anemia liver extract) to a synthetic milk diet resulted in an increased growth rate in baby pigs over an 8-week period. The final weights of pigs receiving Reticulogen averaged 19.96 and 21.6 kg as compared with 13.53 and 16.0 kg for those not receiving Reticulogen in two comparisons.

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Effect of a Progesterone Compound on Growth of a Transplanted Granulosa Cell Tumor.*

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Progesterone has been considered to be inhibitory to, or without effect on, tumor growth. Heimann[1] reported a marked decrease in incidence of mammary carcinoma in the RIII strain of mice treated with progesterone alone and a still lower incidence when this was combined with testosterone. He stated, however, that it did not prevent growth of transplanted tumors in mice. He also found[1b] that it inhibited growth of adenomatous portions of breast fibroadenomata and decreased the number of takes of auto- and homo-transplants in rats. There was no effect on fibroma, myxoma, or sarcoma or on adenomatous growth in pregnant or castrated rats. In the guinea pig Lipschütz and Vargas[2] observed decrease in size, and actual disappearance, of the fibroids caused by oestrogen. Burrows and Hoch-Ligeti,[3] however, found no change in incidence of mammary carcinoma in mice injected weekly with 1 mg of progesterone, and Loesser[4] found slight, if any, improvement in animals or patients with breast carcinoma treated with progesterone.

The effects of larger amounts of progesterone on tumor growth have been studied, using a material containing a high concentration of progesterone.§ Preliminary experiments indicated a growth-stimulating effect on mammary carcinoma in mice.[5] A study of the effect of this material on a transplanted ovarian tumor was then undertaken. Subcutaneous transplantation of a granulosa cell tumor induced by intrasplenic transplants[6] in mice of the C57 strain had been attempted.

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‡ Anna Fuller Fund Fellow. Present address: Hsiang-ya Medical College, Changsha, China.

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4 Loesser, A. A., *Lancet*, 1941, **2**, 698.

§ A crude preparation believed to contain 200-250 I.U. Progesterone/cc made available through the courtesy of E. A. Engstrom, The Glidden Co., Chicago, Ill.

5 Cliffton, E. E., unpublished data.

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