normal persons including fetal, children, young adult, and aged representatives.

The serum polysaccharide (both non-glucosamine and glucosamine) was lowest in the fetal group and highest in the aged representatives, the children and young adult groups being intermediate, thus showing a tendency to increase with age.

Concentrations of serum polysaccharide (both non-glucosamine and glucosamine) for young adults were significantly higher than those of fetal serums, and significantly lower than for the aged. The total protein for young adults was significantly higher than in fetal sera. Significant positive correlations were found between non-glucosamine polysaccharide and total protein and also between non-glucosamine polysaccharide and glucosamine.

In the same individual, non-glucosamine serum polysaccharide and glucosamine were subject to about the same variation as serum protein over a period of sixteen months. Small variations in diet appeared to have little effect. No appreciable changes occurred in serum polysaccharides during the menstrual cycle.

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Failure of Trypan Red to Protect Against Certain Neurotropic Viruses ("M.M." and Russian Spring-Summer Encephalitis).*

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Investigations by Aird[1] on brilliant vital red and by Aird and Strait[2] on trypan red have shown these supra-vital dyes to affect the distribution of intravenously injected cocaine within the central nervous system. This was presumably due to an alteration of the permeability of the blood-brain barrier since the amount of cocaine reaching the cerebral cortex of cats receiving the dyes was decreased by 31% as compared with the controls not receiving the dyes. As a result of these studies and others, the question was raised as to the possible protective effect of these substances against infection of the central nervous system by neurotropic viruses. Due to the interruption of our studies by the late war we were unable to engage in experimental work along these lines.

Wood and Rusoff[3] claimed to demonstrate the protective effect of trypan red on the "M. M." virus.[4] Unfortunately, Wood and Rusoff inoculated the dye repeatedly by the intraperitoneal route and subsequently inoculated the virus by the same route. It has been the general experience of bacteriologists and immunologists that a local, non-specific protection against most infectious agents is set up in the peritoneal cavity by many types of inert substances when given previously by the same route.

Further studies on the possible protective effect of the supra-vital dyes against virus infections of the central nervous system, therefore, appeared indicated. Considering the mode of action of the dyes, it seemed wise to select viruses capable of infecting the central nervous system when inoculated by a peripheral route, as opposed to viruses requiring direct inoculation into the central

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2 Aird, R. B., and Strait, L., *Arch. Neurol. and Psychiat.*, 1944, **51**, 54.

3 Wood, H. G., and Rusoff, I. I., *J. Exp. Med.*, 1945, **82**, 297.

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TABLE I.
Effect of Trypan Red on Russian Spring-Summer Encephalitis Virus in Mice.

Date of experiment	No. of mice	LD_{50} controls	LD_{50} trypan red	LD_{50} Difference
8/27/46	60	5.75	5.75	0.00
1/20/47	78	5.76	6.44	+0.68

TABLE II.
Effect of Trypan Red on "M.M." Virus in Mice.

Date of experiment	No. of mice	LD_{50} controls	LD_{50} trypan red	LD_{50} Difference
5/23/47	47	5.63	5.52	—0.11
23	47	6.42	6.38	—0.04
7/16	80	6.08	5.68	—0.40
10/25	189	6.48	6.33	—0.15

nervous system. Our experiments were, therefore, planned to employ the same virus used by Wood and Rusoff, and in addition the Russian spring-summer encephalitis virus (Sophy strain-eastern type).† Both of the viruses infect readily by the intraperitoneal and other peripheral routes. The route or mode of invasion of the central nervous system, however, is as yet unknown for both viruses. It is entirely possible that they are not carried by the blood stream (as is cocaine) but may invade through peripheral nerves.

Mice were inoculated repeatedly as indicated below, with 1% trypan red in saline by the subcutaneous route over a period of several days. This resulted in their skin assuming a bright reddish tint. Controls were given saline in the same dosage. Following this preparation all mice were inoculated with one or the other of the viruses by the *intraperitoneal* route. Serial dilutions of the virus were prepared from frozen ampoules and four 10-fold dilutions were selected to cover the range of virus from that which would kill all mice to that which would not kill any. The LD-50 was calculated by the method of Reed and Muench.[5]

Russian Spring-summer virus. The mice were prepared by 7 subcutaneous inoculations of 0.1 to .05 cc of 1% trypan red or with saline over a period of 4 weeks. Five days after the last injection the virus was inoculated intraperitoneally. The results of 2 consecutive experiments employing 138 mice are shown in Table I. It will be noted that, if any effect was produced, the mice inoculated with the dye were the more susceptible. In our opinion, however, the difference shown is not significant.

"M. M." virus. In these experiments less dye was given. The dosage given by Wood and Rusoff was followed exactly: 0.1 cc of a 1% solution being injected on 3 successive days. The virus was given one day after the last dye injection. As in all our tests the dye was given subcutaneously and the virus intraperitoneally. The results of 4 successive experiments employing 363 mice, are shown in Table II. It may be observed that the differences between the controls and those inoculated with dye ranged from 0.11 to 0.40 log and never attained a significant difference. However, in each experiment the small differences which did appear always occurred in the same direction; a lower LD-50 in those receiving the dye. It was felt that this effect, however, could well be due to chance alone.

Conclusions. Trypan red failed to produce a significant protection against Russian Spring-summer encephalitis virus and "M.M." virus when the dye was injected subcutaneously and the virus was inoculated by the intraperitoneal route. These experiments fail to confirm the results of others who injected both dye and virus intraperitoneally. We in-

† Obtained through the courtesy of the Army Medical Department Virus and Rickettsia Laboratories.

[5] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

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.

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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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9 *Ibid.*, *J. Animal Sci.*, 1948, **7**, 486.

10 Grau, C. R., and Almquist, H. J., *J. Nutrition*, 1943, **26**, 631.

* 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.