are more adequate for the growth of chicks than purified casein alone. Other workers have shown that the chick has a greater requirement for arginine,[10] glycine,[11] and cystine[12] than the rat. Apparently powdered hoofs supply these amino acids in greater amounts than casein. Therefore, the deficiencies of powdered hoofs in the nutrition of the rat may be due to amino acid imbalance rather than indigestibility. However, the latter possibility cannot as yet be eliminated entirely since the digestive tract of the chick may be more capable of digesting material of this nature than the tract of the rat.

Although gizzard lesions were only observed in those chicks receiving powdered hoofs, it appears from other work performed with practical poultry diets that the lesions are not specific for powdered hoofs.

Conclusions: Rats and chicks are able to utilize powdered pig and hog hoofs as a source of protein for growth. The available protein of powdered hoofs is more adequate for the growing chick than for the growing rat.

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13990

Titration and Neutralization of the Western Strain of Equine Encephalomyelitis Virus in Tissue Culture.*

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Titration of virus potency and demonstration of viral neutralizing antibodies are procedures not only involving large numbers of animals but also presenting disadvantages which make quantitative interpretation difficult. In an effort to evolve a simplified approach and particularly to remove the variable of individual animal reactivity, tissue culture methods were utilized for titration and neutralization of the Western strain of equine encephalomyelitis virus (W.E.E.). The method is presented here. The principle on which the study was made possible is based on the finding that tissue failed to grow when it was heavily infected with the virus.

In Vitro Titration. Ten-fold dilutions of W.E.E. virus were made in a buffered salt solution. Instead of injecting the virus into animals, one drop of the material from each dilution was inoculated into tissue cultures consisting of a series of tubes each containing 10 pieces of minced skeletal muscles from a 9-day developing chick embryo and 1 cc of serum ultrafiltrate diluted in buffered salt solution.[1] The different mixtures were incubated at 37.5°C. At the end of 48 hours of incubation, pieces of tissue from each tube were transferred and patched with plasma in micro-culture slides. They were then kept at 37.5°C and the readings were made under the low power microscope 48 hours later. It was found that cells which were not infected (or had overcome the infection) grow out in the plasma patch. Growth was abundant and sheets of fibroblasts were clearly visible. Contrariwise, when virus was present, no growth from the explant was observed.

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TABLE I.
Comparison of *in vitro* and *in vivo* Titration of W.E.E. Virus.

Titrated in	Virus dilution tested							
	No virus	10-2	10-3	10-4	10-5	10-6	10-7	10-8
Mice	A A A	2 2 2	2 2 2	2 2 2	2 3 3	A A A	A A A	A A A
Tissue culture	G G	0 0	0 0	0 0	0 0	0 0	G G	G G
	G G	0 0	0 0	0 0	0 0	0 0	G G	G G

A = Mouse remained alive 5 days.
Each number indicates day of death of the animal after the infection and presumably represents a virus death.
G = Growth of fibroblasts from explants noted.
0 = No growth from the explants.
Each "G" or "O" represents growth activity of a single explant. Four explants were used for each plasma preparation.

To prove that the tissue was really infected after this period of incubation, the supernatant fluid of the cultures inoculated with different dilutions of the virus was injected intracerebrally into mice soon after the pieces of tissue had been removed from the tubes to the plasma preparations. As was to be expected whenever the tissue was infected, the virus was detected in the supernatant fluid by mouse test.

The sensitivity of the *in vitro* titration method presented here was compared with intracerebral mouse inoculation and the results shown in Table I indicate that the titre is higher in the *in vitro* test than in the *in vivo* experiment.

The test was repeated 3 times with similar results.

In Vitro Neutralization. A volume of 10-fold dilutions of the virus was mixed with an equal amount of hyperimmune anti-W.E.E. horse serum prepared by Lederle Laboratories, Inc., New York, N.Y. For control, anti-meningococcus horse serum was used. The serum-virus mixtures were incubated in the water bath at 37.5°C for 30 minutes. Two drops from each mixture were then introduced into separate tubes each containing about 15 pieces of tissue. The serum-virus mixtures and tissue were incubated at 37.5°C for 15 minutes in order to allow the virus to get into the cells. The tissue was then washed with buffered salt solution for 3 times, each time with 5 cc of the solution. The washed tissue from each tube was then transferred into separate tubes each containing 1 cc of dilute serum ultrafiltrate. The entire preparation was incubated at 37.5°C. At the end of 48 hours of incubation the tissue was patched with plasma and returned to the incubator. Readings were made 48 hours later under the low power microscope.

Again the activity of the virus in tissue culture was at all times compared and confirmed by mouse inoculation. The results of the comparison of the *in vitro* and the *in vivo* neutralization experiments are shown in Table II. It can be seen that the amount of neu-

TABLE II.
Comparison of the *in vitro* and *in vivo* Neutralization Tests with W.E.E. Virus.

Serum used	Titrated in	Dilution of the virus tested							
		10-0	10-1	10-2	10-3	10-4	10-5	10-6	10-7
Immune	Mice	2 2	A A	A A	A A	A A	A A	A A	
		2 2	A A	A A	A A	A A	A A	A A	
	Tissue culture	00000	00000	GGGGG	GGGGG	GGGGG	GGGGG	GGGGG	
		00000	00000	GGGGG	GGGGG	GGGGG	GGGGG	GGGGG	
Control	Mice			2 2	2 2	2 2	A A	A A	A A
				2 2	2 2	3 3	A A	A A	A A
	Tissue culture			00000	00000	00000	00000	GGGGG	GGGGG
				00000	00000	00000	00000	GGGGG	GGGGG

Legends as in Table I except that 10 explants were used for each plasma preparation.

tralization (10,000 neutralizing doses) in the *in vitro* experiment is the same as that observed by animal inoculation. Apparently the scale of sensitivity for the detection of virus in both the titration and neutralization experiments has shifted one dilution higher than the *in vivo* tests. The possibility must also be considered that the shift in neutralization activity measured by the *in vitro* method may to some extent be a manifestation of reactivation of neutral mixtures by dilution.[2] Further study of *in vitro* neutralization technic should clarify this point.

Improvement of technical details is being attempted and will be further described.

Conclusions. Titration of potency of W.E.E. virus and quantitative estimation of neutralizing antibodies in hyperimmune horse serum have been demonstrated by use of tissue cultures.

The *in vitro* method of detecting the presence of virus appeared to be more sensitive than intracerebral mouse inoculation.

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13991

Choline Esterase Content of Tissues Without Innervation (the Placenta).

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The probable importance of the choline esterase-acetylcholine system to transmission in the nervous and neuromuscular system suggested that the choline esterase content of the placenta, an organ without nervous tissue (Schmitt[1]) should be investigated. Other tissues without innervation are the blood and the denervated muscle. The choline esterase content of denervated muscles decreases for some weeks after denervation but does not disappear completely as shown in the rat by Martini and Torda[2,3] and Meng[4], in the dog by Martini and Torda[5], in the frog by Feng and Ting[6], in the rabbit by Leibson[7] and in the guinea-pig by Couteaux and Nachmansohn.[8]

The human placenta has a rather high acetylcholine content (Chang and Gaddum,[9] Chang and Wong[10], Chang,[11,12] Reynolds and Foster,[13] Cattaneo[14]). Changes in acetylcholine sensitivity (Euler[15]) and acetylcholine content (Chang,[12,16], Chang, Lee, Meng and Wong[17]) of the placenta during different experimental conditions suggest that this tissue contains choline esterase.

Method. Some minutes after normal delivery the human placenta was perfused through the umbilical vein until all the blood was removed. The choline esterase content of 100 mg finely ground tissue was determined

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