diet. This explanation, however, disregards the important fact that estrus disappeared even in cirrhotic rats as soon as the estrone pellet was completely absorbed. Lack of estrone should not influence the pseudo-estrus of vitamin A deficiency.

Summary. Impairment in ability to inactivate estrone accompanies dietary hepatic injury and can be rectified by the addition of lipotropic factors such as methionine or protein digests.

Fivefold increase of the basal vitamin B supplements was ineffective.

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Studies on Toxicity Complement-Fixing and Immunogenic Activity of Typhus-Infected Yolk Sacs.

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The use of the yolk sac of the developing chick embryo for cultivating typhus rickettsiæ[1] led to the development of vaccines prepared from infected yolk sacs by phenol precipitation[2] and ether extraction[3,4] and to the demonstration of a labile toxic substance in yolk sac cultures of typhus rickettsiæ which was lethal for mice and which was associated with the rickettsiæ themselves.[5,6]

It has been shown that both the complement-fixing activity and the rickettsial content of typhus vaccines were related to the time of harvest of the constituent yolk sacs with reference to the peak of embryo deaths.[7] However, it was also observed that the presence of numerous rickettsiæ did not necessarily indicate toxicity[6] and that the antibody response of guinea pigs, commonly used as a measure of antigenicity, is subject to considerable variations.[8] It was of interest, therefore, to compare complement-fixing activity with the toxicity of yolk sac suspensions and with the antigenicity and rickettsial content of typhus vaccines.

Methods. Eggs in the 7th day of embryonic development were inoculated into the yolk sac with the Breinl strain of epidemic typhus according to the method of Cox.[1] The eggs were candled daily and all yolk sacs harvested were frozen immediately and stored at −70°C until used. All vaccines were prepared by the ether extraction method[4] using a standard procedure (*cf.* [7]).

Complement-fixation tests were done according to the method of Bengtson.[9] Vaccines were titrated with 2 units of hyperimmune guinea pig serum and the titer was recorded as the highest dilution of vaccine showing complete (4+) fixation.

Toxicity titrations were performed as follows: 0.5 ml of serial 2-fold dilutions of the various yolk sac suspensions was injected into

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TABLE I.
Comparison of Toxicity with the Complement-Fixing Activity of Infected Yolk Sac Suspensions.*

Interval between inoculation and harvest	No. of yolk sacs represented	Complement fixation test suspension diluted: 1–16	1–32	1–64	Toxicity titration suspension diluted: 1–2	1–4	1–8	1–16
L-5	15	0	0	0	0/3†	0/4	0/4	
L-6	15	3+	1+	±	4/4	4/4	0/4	0/4
L-7	15	4+	2+	±		4/4	1/4	0/4
L-8	15	4+	4+	2+			4/4	0/4

* 10% tissue suspensions by weight.
† No. of mice dead/ Total No. of mice.
L-5 = Embryos living 5 days after inoculation.

the tail vein of mice.† The number of deaths was recorded 18 hours after inoculation.

The approximate rickettsial content of a vaccine was estimated by microscopic examination of films of vaccine made with a standard loop and stained by Macchiavello's technic (*c.f.* [7]).

Antigenicity was measured by immunizing† mice as follows: Method A: Each of 30 mice was given one intraperitoneal inoculation of 0.2 ml of undiluted vaccine. Method B: Groups of 10 mice each were given one intraperitoneal inoculation of 0.5 ml of vaccine diluted 1-5, 1-25, and 1-125 respectively. Fourteen days after vaccination all vaccinated mice were challenged with 0.5 ml of toxic substance representing 2-4 LD_{50} injected into the tail vein. The results were recorded 18 hours after challenge.

Experimental. Comparison of toxicity with complement-fixing activity of infected yolk sac suspensions. Inasmuch as both the rickettsial content and complement-fixing activity of vaccines were highest when the constituent yolk sacs were harvested from embryos still living at the peak of embryo deaths,[7] yolk sacs were harvested only from living embryos to prevent loss of toxicity following death of the embryo. In comparing toxicity with complement-fixing activity yolk sacs were harvested from 15 living embryos on the 5th, 6th, 7th, and 8th day after inoculation respectively. Each pool of yolk sacs thus obtained was frozen immediately and stored at −70°C. Approximately one week later 10% untreated tissue suspensions in saline were prepared from each pool of yolk sacs and each suspension was titrated for complement-fixing activity and for toxicity in mice. It will be seen from Table I that the complement-fixing titer of yolk sac suspensions increased from less than 1-16 in the case of suspension L-5, prepared from embryos harvested 5 days after inoculation, to 1-32 in the case of suspension L-8, prepared from embryos harvested 8 days after inoculation. When the same suspensions were titrated for the presence of toxic substance by intravenous injection of mice suspension L-5 diluted 1-2 was not lethal for mice whereas suspension L-8 diluted 1-8 was lethal for all mice inoculated. It is thus evident that the complement-fixing activity and toxicity of untreated saline suspensions of infected yolk sacs harvested from living embryos rose together.

Comparison of antigenicity with complement-fixing activity and rickettsial content of typhus vaccines. The complement-fixing activity, rickettsial content and antigenicity of typhus vaccines prepared from yolk sacs harvested before and at the peak of embryo deaths were compared and the results are presented in Table II. It was found that the complement-fixing titer increased from less than 1-8 in the case of vaccine D-5, prepared from embryos dead 5 days after inoculation, to 1-64 in the case of vaccine L-7, prepared from embryos still living 7 days after inoculation and that the rickettsial content of the vaccines increased with the complement-fixing activity. When mice were immunized by Method A only 6 of 28 (21.4%) of the mice immunized with vaccine D-5 were protected whereas 17 of 27 (62.9%) of the mice immunized with vaccine D-6 and 24 of 28 (85.7%)

† Young albino Swiss mice (Webster strain) weighing 12-13 g were used throughout these experiments.

TABLE II.
Comparison of Complement-Fixing Activity, Rickettsial Content and Antigenicity of Vaccines Prepared from Embryos Harvested Before and at the Peak of Embryo Deaths.

		Vaccines prepared from yolk sacs harvested from embryos: D-5	D-6	D-7	L-7	
No. of yolk sacs included in vaccine		90	102	146	65	
Rickettsial content		±	+	++	++++	
Complement fixation test	Vaccine diluted: 1-8	3+				
	1-16	1+	4+	4+	4+	
	1-32	±	3+	4+	4+	
	1-64	0	2+	3+	4+	
	1-128	0	0	0	1	
Antigenicity for mice						
Method A—0.2 ml undiluted vaccine inoculated I.P.						
Vaccinated group						
Total No. of mice		28	27		28	
No. protected		6/28*	17/27	n.t.	24/28	
% protected		21.4	62.9		85.7	
Control group	Challenge dose diluted: 1-2					0/16*
	1-4					10/15
Method B—0.5 ml of serial dilutions of vaccine inoculated I.P.						
Vaccinated group	Vaccine diluted: 1-5	0/9	4/9	n.t.	7/10	
	1-25	0/10	0/10		6/10	
	1-125	0/10	0/10		0/10	
Control group	Challenge dose diluted: 1-2					0/10
	1-4					8/10

D-5 Dead 5 days after inoculation.
L-7 Living 7 days after inoculation.
\+ Five rickettsiae per field.
++++ Over 100 rickettsiae per field.
n.t. Not tested.
4+ Complete fixation of complement.
0 No fixation of complement.
* No. of mice surviving/Total No. of mice.
I.P. Intraperitoneally.

of the mice immunized with vaccine L-7 were protected. When mice were immunized by Method B vaccine D-5 diluted 1-5 did not protect any of the 9 vaccinated mice whereas vaccine D-6 diluted 1-5 protected 4 of 9 vaccinated mice, and vaccine L-7 diluted 1-25 protected 6 of 10 vaccinated mice. In addition to the data presented, in 7 additional experiments the complement-fixing activity of the vaccine likewise followed the antigenicity of the vaccine.

Discussion. It is clear from the data presented that complement-fixing activity followed not only the rickettsial content and antigenicity for mice of vaccines prepared in an identical manner but also the toxicity of yolk sac suspensions when care was taken to preserve toxicity. However, the factors responsible for toxicity and complement-fixing activity are not identical since toxicity is destroyed by formalin and ether[6] while complement-fixing activity is not. Furthermore, the complement-fixing activity of a given yolk sac suspension or vaccine represents not only the rickettsiæ themselves but also a soluble antigen capable of immunizing guinea pigs.[10] Nevertheless, when yolk sac suspensions or vaccines were prepared as described from embryos harvested on consecutive days an increase in complement-fixing activity was always accompanied by an increase in toxicity or antigenicity.

Summary. Under the conditions described, the toxicity of untreated yolk suspensions and the antigenicity of typhus vaccines prepared from embryos harvested on consecutive days rose together with the complement-fixing activity of the same antigens.

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