the vein is prevented, a comparison of the flow from the adrenals in successive observations is made possible by noting the intervals of time necessary for the pocket to fill up to this point. The quantity of blood required to fill the pocket can be determined once for all in each animal. The vertical position of a portion of the pocket helps to empty it without manipulation when the clamp is removed.

6. The sensitiveness of the eye-reactions to epinephrin discharged from the adrenals, for example in response to stimulation of the splanchnics, can be increased notably by temporarily clamping off alternative arterial paths. This must be done at such an interval of time after the beginning of stimulation as is not more than sufficient to allow the epinephrin to reach the beginning of the aorta. A larger proportion of the blood containing the epinephrin is thus forced to take the path to the eye whose reactions are being studied. If, for instance, the left iris is the denervated one, clamping at the proper moment of the thoracic aorta and the innominate markedly increases the reaction. It can be further increased by tying off all accessible branches of the left carotid except those through which the eye must obtain its blood supply.

108 (1172)

Attenuation of the living agents of cyanolophia.

By RHODA ERDMANN. (By invitation.)

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Paschen[1] (1911) says that leucocytes must play an important part in the process of immunization. This remark seems partly justified in the attenuation process of cyanolophia. The living agents of cyanolophia are differently affected in tissue cultures of *red* bone marrow from *white* bone marrow.

[1] Paschen, O., "Handbuch der Technik und Methodik der Immunitätsforschung," 1911.

EXPERIMENT I, 1915.

SERUM B TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN A IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF RED BONE MARROW AND CHICKEN PLASMA.

Length of Time in which Virulent Serum *B* was Cultivated in Tissue Culture at 30° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Nov. 12–Nov. 15	No. 1	Nov. 18	48 hours
Nov. 12–Nov. 15	No. 3	Nov. 17	38 hours
Nov. 12–Nov. 18	No. 2	Nov. 21	72 hours

This proves that the virus can be kept alive six days at a temperature of 38° C. in a tissue culture of *red* bone marrow. Chickens No. 1 and No. 3 died in 48 and 38 hours. Chicken No. 2, which had been inoculated with serum B that had been kept 6 days in the tissue culture, died twenty-four hours later than the chicken which had been inoculated with serum B that had been only *three* days in a tissue culture of red bone marrow. This proves a certain attenuation by the cultivation of virulent serum in *red* bone marrow.

The living agents, which probably cause cyanolophia, can be cultivated in red bone marrow tissue cultures even longer than six days without losing their virulence.

EXPERIMENT XII, 1916.

SERUM V TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN U IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF RED BONE MARROW AND CHICKEN PLASMA.

Length of Time in Which Virulent Serum *V* was Cultivated in Tissue Culture at 38° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Feb. 23–March 2	No. 17	March 4	48 hours
Feb. 23–March 6	No. 18	Remained alive	
Feb. 23–March 11	No. 19	Remained alive	

So we can keep virulent the living agents of cyanolophia outside of the chicken 6–8 days at 38° C., yet in the tissue culture of *red* bone marrow the virus dies after 12 days. This is a perfect analogue to the experiment of Marchoux,[1] who cultivated the virus of cyanolophia in a culture medium which contained red blood corpuscles. He even believed that the living agents of cyanolophia had multiplied and produced a much stronger virus than that

[1] Marchoux, "Cultures in vitro du virus de la peste aviaire," *Compt. rend. Acad. Sc.*, T. 147, p. 357, 1908.

he inoculated in his cultures. In tissue cultures of red bone marrow no multiplication of the virus was observed, but a certain attenuation, as proved by the prolongation of the incubation period.

Different results were obtained by using *white* bone marrow.

EXPERIMENT II, 1915.

SERUM C TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN B IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF WHITE BONE MARROW AND CHICKEN PLASMA.

Length of Time in Which Virulent Serum *B* was Cultivated in Tissue Culture at 38° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Nov. 30–Dec. 4	No. 3*a*	Living	
Nov. 30–Dec. 6	No. 4	Living	

SERUM C, THE SAME AS USED IN EXPERIMENT BEFORE, IN RED BONE MARROW AND PLASMA.

Length of Time in which Virulent Serum *C* was Cultivated in Tissue Culture at 30°C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Nov. 30–Dec. 6	No. 5	Dec. 9	72 hours

This experiment proves that after 4 or 6 days in white bone marrow the virus is attenuated. After inoculation of virus in red bone marrow the animal died. A controlling experiment, in which serum C was used, which was kept 7 days on ice, showed that this serum killed the chicken after 38 hours, the usual time in which this strain of cyanolophia killed the animal. It was perfectly attenuated by the cultivation in white bone marrow, partially by cultivation in red bone marrow, and not at all by keeping the serum on ice.

The inoculation of attenuated serum and white bone marrow protected, to a certain degree, the chicken against a new inoculation, as the following experiments prove.

EXPERIMENT III, 1915–1916.

SERUM Y TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN H IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF WHITE BONE MARROW AND PLASMA.

Length of Time in which Virulent Serum *y* was Cultivated in Tissue Culture at 38° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Dec. 26–Dec. 29	No. 3*a*	Living	
Dec. 26–Dec. 29	No. 4	Living	
Dec. 26–Dec. 29	No. 6	Died Jan. 1	72 hours

Chickens Nos. 3*a* and 4 had been inoculated with serum B and white bone marrow before, but not chicken No. 6. The same results were attained in experiments IV, V, VI, VIII, and X. All chickens which were treated with attenuated serum did not die when inoculated with a 2d or 3d dose of attenuated serum. All chickens which were not treated succumbed to the first doses of attenuated virus when it was kept only 2 or 3 days' time in tissue culture of white bone marrow. Always controlling experiments with the same untreated serum kept on ice were started, which killed the animals in due time.

It is possible to keep virulent the living agents of cyanolophia in plasma alone at a temperature of 38° for six days and longer, but the *same* virus dies in plasma, in which living white bone marrow is kept, in six days (experiment IX). The controlling experiment with serum, which was kept on ice, was positive, so it is true that the virulence of living agents of cyanolophia will be attenuated, and later die through the activity of the leucocytes. It was possible to shorten the length of time in which virulent serum was kept in tissue cultures of white bone marrow, and still inoculate it without success when the *treated animals were used again.* Animals 3*a*, 4, 7 and 8 survived after inoculation with serum M_1 which had been only 2 days in tissue culture (Experiment VI). Serum M_1 killed chicken M_2 after it was kept 5 days on ice, in due time. A shortening of the attenuation period to twenty-four hours was not sufficient to weaken the serum M_2. Chicken 7, which again was used, died in forty-eight hours, after having veen inoculated with serum M_2, that had only been one day in plasma and white bone marrow.

Steinhardt and Lambert[1] cultivated the living agents of vaccinia in tissue cultures of rabbit cornea. They report a definite increase of the virus, as measured by the effects of successful reinoculations. Growth of the virus could be observed in tissue cultures of the rabbit's cornea only, while heart, kidney and liver gave no results. My experiments, previously reported, prove a rapid attenuation of the virus in *white* bone marrow tissue

[1] Steinhardt, E., and Lambert, R. A., "Studies on the Cultivation of the Virus of Vaccinia, II," *Journ. of Inf. Diseases*, 1914, Vol. 14, pp. 87–92.

culture. This is quite remarkable, because the living agent of cyanolophia is not surpassed in virulence by any other virus.

The next series of experiments will deal with the attenuation of the living agents of cyanolophia in brain and liver tissue cultures and with the importance of these and the white bone marrow tissue cultures for active immunization.

109 (1173)

Experiments on the physiology of digestion in Blattidæ.

By ELDON W. SANFORD. (By invitation.)

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The question as to whether fat is digested and absorbed in the crop of the cockroach was answered in the affirmative by Professor Petrunkevitch in 1898, but in the negative by more recent authors. My investigations, which were done under the direction of Professor Petrunkevitch, show that fat is split to soluble products and absorbed in large amount in the crop, the process being observable as gradually more and more in the crop's epithelial cells at successive intervals up to forty-eight hours, and gradually less afterward. Some cells absorb so much that they appear solid black when stained with osmic acid. Ligation of the crop from the stomach does not hinder or modify the process. Fatty acids are absorbed like fats.

At certain intervals after fat feeding much fat is found in the tracheal tubes, sometimes filling them, sometimes in a thin layer on their walls, sometimes only on the supporting spirals, and sometimes mingled with chyme. This chyme resembles that normally present in the crop lumen; it is regularly present in some of the tracheæ, and in it leucocytes are often found. The chyme is evidently a normal content. The fat enters the tubes through the tracheal end cells, after being absorbed by them from the lumen of the crop.