93 (1271)

Immunization against cyanolophia.

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When in June, 1915, I was introduced to the study of *Cyanolophia* by A. von Wassermann, Berlin, I performed, under his guidance, several series of experiments to immunize against this chicken disease caused by a filtrable virus. We tried to establish active immunity by treating the animals with attenuated inoculated brain, attenuated inoculated liver, attenuated serum inoculated into bone marrow and virulent sera of different ages and different strengths. The attenuation was effected either by "cultivating" the virulent tissues in chicken plasma after the tissue culture method or by weakening the virulent serum by "cultivation" in bone marrow and chicken plasma. Our tentative experiments, from June, 1915, to October, 1915, did not give results. Only one chicken resisted an inoculation of virulent brain after the following treatment. This chicken, No. 13, had received two inoculations with serum which was obtained 17 hours after the inoculation of virulent brain into another animal. We applied one inoculation with attenuated brain tissue and later bone marrow with attenuated serum was implanted under the skin. After a short interval this animal received another serum treatment and then two days later a lethal dose of virulent brain. It survived this strongest test, while a control chicken which was inoculated with the same brain, but had not undergone these different treatments, died in due time. The immunity of No. 13 was gained without intention. Owing to the scarcity of chickens to be used for experimental purposes at this time in Germany, we had always used the same chickens for our experiments, after the preceding inoculations with the different attenuated materials proved ineffective. So we did not expect No. 13 to live after we inoculated it with virulent brain. We intended it to die for affording virulent serum. As closely as possible we repeated the same treatment, which we had applied to No. 13

by chance, with four other chickens but without success. We could only note a retarding of the incubation period. But with serum No. 13 we could gain passive immunity of an untreated chicken, No. 21.

At this stage of my work I left Germany and continued my experiments in this country at the Osborn Zoölogical Laboratory of Yale University, from October, 1915, up to the present time.

My problem was to find out which of these various factors von Wassermann and I had used in Germany were necessary to produce active immunity, which were not necessary and could be omitted, and which new factors had to be added to produce immunity—not by chance but by a graded series of experiments. It was not necessary to try to gain immunization with serum treatment alone because these experiments had been made by various authors. (See Maggiora and Valenti(1).) Also it was not necessary to try to gain immunization by a treatment with virulent brain alone which had been attenuated by staying long periods in glycerine. (See von Prowazek (2).) Nor did it seem advisable to try to gain immunization by applying to the chickens doses of desiccated brain alone. (See Kraus (3, 4, 5).) My own experience, which I reported here in May, 1916, in trying to immunize with attenuated serum and bone marrow tissue culture alone had not been successful. I could only show that the incubation period could be prolonged. It was certain that our known methods of immunization ought to be varied or combined because none of them alone produced results. Only the knowledge that there is an active immunity induced me to go on in a rather empirical way guided by the idea that it must be possible to raise the resistance of the chicken against *Cyanolophia* by slight, nearly unnoticeable attacks of the disease.

From October, 1915, to July, 1916, I inoculated chickens with desiccated brains on a large scale. These brains[1] had been taken from chickens which died of *Cyanolophia* after a *very prolonged* incubation period. Of these brains I had six which are called in the following Table No. 1, Des. 1 to Des. 6. Table 1 gives an exact survey of the chickens and shows how often each one had been inoculated with the different desiccated brains.

[1] N. B. For details concerning the method of preparing the different protective materials, the exact amount of them, etc., see the main paper.

TABLE I.

Des. 1.	Des. 2.	Des. 3.	Des. 4.	Des. 5.	Des. 6.
11**	19	15****	28	15	15**
13** died	40	19 died	29	28	30**
15	69	24	30	29	31**
16	71	25	32	32	32**
29		28	33	33	33**
30		29**	34	34	40
50		30	40**	54	54**
68		31	46	55	55**
83		32**	54	65**	65**
84		33**	55**	67	67**
85		34**	65	68*	68**
87		40**	67	70***	70
89		41	68	72**	72
		43	70	75	75
		45	72	76	77
		46	75	77	79
		54***	76	78	80**
		55***	77	79	81**
		65**	78	80	82**
		67	79	81	83**
		68	80	82**	86**
		70	81		88**
		72	82		89
		73	84		90
		74	85		91
		80	86		96
		81	87		97
		87	88		98
			89		99
					100

The same chicken, or different ones, had meanwhile been treated with serum, which had been attenuated in bone marrow growing in a plasma medium (Table II).

TABLE II.

CHICKENS TREATED WITH SERUM ATTENUATED IN BONE MARROW.

Ser. B.	Ser. C	Ser. I.	Ser. M.	Ser. N.	Ser. O.	Ser. T.	Ser. V.	Ser. H_1.
1 died	3a	3a	3a**	3a	9	3a		28
2 died	4	4**	4	4	10 died	4		29
3 died	5 died	6 died	7**	7 died		3		30
		7**	8	8			17** died	31
		8					18	32
							19	33
								34
								40
								54
								55
								65
								68

Also during this time, the same or different chickens were

inoculated with brain tissue which had been during shorter or longer periods in a plasma medium. Table III gives the exact

TABLE III.

O.	X.	No. 47.
11	11	11
12** died	22 died	56 died
13	23 died	57
14	24	58
	25	59
	26 died	60
	27 died	61
	28**	62
	29	63
	30	64
	31	65
	32	
	33	
	34	
	35 died	
	36	
	37 died	
	38	
	50	

numbers and shows how often these chickens had been inoculated with virulent brain in a plasma medium.

A few chickens were treated with virulent sera which had been kept on ice (Table IV).

TABLE IV.

Serum O.	Serum T.	Serum Q.	Serum R.	Serum Z.
24	24	24	52 died	67
25	25	25		73
28	28			74

From the chickens prepared in this prescribed manner, I chose from the different groups several animals. For example: No. 15 and No. 77 had received only desiccated brain, No. 15 nine times and No. 77 six times. Both died, together with control animal No. U_1, after being inoculated with virulent brain No. Q. No. 11 and No. 68 which had been treated with desiccated brain and with brain which had been attenuated in a plasma medium, died, together with control animal S_1, after application of virulent brain No. Q. That proves that continued doses of desiccated brain and of brain attenuated in a plasma medium do not protect the chicken against *Cyanolophia*.

The combination of attenuated serum in bone marrow and desiccated brain also did not prove protective. Chickens No. 54 and No. 55 died after inoculation with virulent brain Q. Also a combination of attenuated serum and attenuated brain in a plasma medium proved to be a failure (Chicken No. 24) just as well as the combination of attenuated serum and desiccated brain (Chicken No. 73). But in many cases the incubation periods were prolonged. This caused me to believe that in some of my animals I had raised the resisting power, as Chickens 11, 15, 65, and 67 proved which resisted so many treatments. (Compare Table I and Table II.) Before Chickens 11, 15, and 67 underwent their final test, I applied sera of these more resistent chickens to all animals which were left and some of which had not been treated so frequently with attenuated material. Then I raised the resistance again by the following careful treatment. Chickens 30, 31, 32, 33, 40, 57, 65, 70, 80, 81, 84, 86, 87, 88, 89, 90, 91, 96, 97, 98, 99, and 100 were now treated successively between the 3d and 29th of November, with virulent brain, virulent liver, and virulent serum, which had been allowed to grow with embryonic chicken tissues in a plasma medium. When after these new protective treatments all the chickens were again tested, brain Q being inoculated in all of them, only chickens 57, 84, and 88, died, together with control animal A_2. Brain Q killed a normal untreated chicken, after it had been nine months in glycerine, in three to five days. This is not a very virulent brain. But having attained immunization of 22 chickens against a not too virulent brain, the possibility of immunization against a highly virulent brain was given. But at first I made a mistake and took the highly virulent brain No. 15 which had been only one month in glycerine, and applied serum No. 65 and brain No. 15 to chicken No. 100 and lost it by this too severe treatment. Therefore I used at first for the rest of the chickens the less virulent brain No. 21. I prepared them by an inoculation with serum No. 65 and brain No. 21 (which had been in glycerine nine months and killed a normal chicken in between three and four days) to all of them together with a control animal No. K_2. This control animal died together with 86, 87 and 98. I waited now several weeks for the final test. Then all the chickens underwent a renewed

application of serum No. 65 and brain No. Q, and several days later serum No. 65 and brain No. 15 together with control animal O_2. I lost chickens 96 and 99 and O_2. So I kept 8 immune chickens, because I used four of them for experiments which I cannot record here. Chickens 30, 31, 32, 33, 40, 65, 80 and 81 remained, and passive immunity could be easily produced by using, for example, the serum of either 40 or 65. Both sera applied to untreated chickens together with virulent brain No. 21 or No. 15 protected them fully. One chicken four weeks after its first treatment with serum 65 and brain No. 21 was not killed by a renewed application of brain No. 21. So at least the passive immunity lasted four weeks. I could not follow my experiments to an end because I was compelled by circumstances beyond my control to kill the immune chickens on the 23rd of April, 1917. This causes me to publish my results as they are:

They prove that it is possible to raise the resistance against *Cyanolophia* by slow degrees in applying to the animals the attenuated virus. But, and that seems important, the virus must occur in different physiological stages in the body, as already von Prowazeck believed, and the animal has to be protected against all these possible stages of the agent. The form of the agent in serum, in brain and liver tissue and that form which appears when virulent brain and liver tissue or serum is attenuated with embryonic tissue, must be physiologically different, and it seems only logical to immunize against each of these supposed forms.

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