STUDIES ON THE RELATIONSHIP OF THE SEX HORMONES TO INFECTION

I. The Effect of the Estrogenic and Gonadotropic Hormones on Vaccinia and the Spreading Factor

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In 1932 we reported (1) that pregnancy in rabbits altered the reactivity of the tissues to the virus of infectious myxomatosis, the secondary lesions being larger and the primary ones smaller in the pregnant animal than in the controls. As viruses are noted for their intimate parasitism it was thought that further study along this line would yield valuable information in regard to the host in the host-virus complex. Since pregnancy is a complex phenomenon we thought it desirable to try to obtain similar results by using only a few of the factors involved. It was thought that this could best be done by using some of the pregnancy hormones. In this paper we are reporting results which show that the estrogenic hormone, and perhaps also the gonadotropic hormone increase the resistance of the castrated rabbit to vaccinia and also that they inhibit the spread of India ink in the dermal tissues.

In the paper referred to above we reviewed much of the literature concerning the effect of pregnancy on the growth of tumors and resistance to infection. Many of these papers are contradictory and only a few of the most outstanding ones will be mentioned here. Bainbridge (2) in a summary of most of the work on tumors concludes that pregnancy stimulates the growth of most tumors with the exception of epitheliomas. Williams (3) in discussing the effect of pregnancy on infections concludes that with the exception of scarlet fever there is little to support the hypothesis that pregnancy increases the resistance of the body to infection. Since 1932 there have been several papers dealing with the effect of pregnancy or the sex hormones on infection. Pearce and her associates (4) showed in a large number of rabbits that pregnant animals were more resistant to vaccinia infection than non-pregnant ones and that females were more refractory than

males. Aycock (5) reported that the estrogenic hormone protected 5 out of 6 castrated monkeys against poliomyelitis. Kemp (6) reviews the rather extensive literature on the effect of the female sex hormones and pregnancy on the course of syphilis. He agrees with preceding workers that pregnancy modifies the course of syphilis, but was not able to confirm the work of others that this could be accomplished with estrogenic hormones.

In addition to this work on the effect of pregnancy on tumors and infection, there are a number of papers dealing with the production in various allergic states of changes associated either with pregnancy or resulting from the injection of the various sex hormones. Williamson (7) reports 13 patients who had less frequent attacks of asthma during pregnancy and 14 sensitive to pollen who showed no change during pregnancy. Manwaring (8) states that both pregnancy and parturition make it more difficult to sensitize a guinea pig.

Recently Solomonica and Kurzrok (9) reported that if anterior pituitary extract were given from 7 to 28 days before the guinea pigs were sensitized, it resulted in a modification of anaphylactic shock. This extract, if given after the animal was sensitized, had no effect in modifying anaphylactic shock. Amniotin, Squibb, when given after the animals were sensitized, caused a modification of anaphylactic shock. The effect of this preparation before the sensitizing period was not tried.

In an experimental study of anaphylactic shock in a large number of guinea pigs, we obtained results which suggested that the estrogenic hormone increased the susceptibility of the guinea pig to sensitization with a foreign protein, while the gonadotropic hormone made them more difficult to sensitize. There was, however, so much variation in the controls that a different method of approach seemed indicated. In addition to these papers Bray (10) cites a number of others which show a relationship between allergic manifestations and the female sex hormones.

Methods and Materials

Animals.—Young castrated adult male rabbits weighing about 2 kilos were used.

Operative Procedures.—The rabbits were castrated aseptically under ether anesthesia. This was done by making a small incision on both sides over the spermatic cord. The cord was freed and a suture placed around it. It was then cut and the testis and epididymis removed from the scrotal sac, after which the incision was sutured. The animals were kept for 3 weeks after the operation before the endocrine injections were started.

Endocrine Preparations.—The estrogenic preparation used was estrone and the gonadotropic preparation was anterior pituitary—like gonadotropic hormone of pregnancy urine.[1] The rabbits in the first experiment receiving the estrogenic

[1] Both of these were kindly supplied by Parke, Davis as theelin in oil and antuitrin S.

hormone were given 1000 international units per day for 24 days and the rabbits in the second experiment 2000 international units for the same period of time. The rabbits receiving the gonadotropic hormone were given daily injections of 400 rat units in the first experiment and 200 in the second experiment. All injections were given subcutaneously. The control animals were given 2 cc. of physiological saline daily.

Virus.—The virus used was the Levaditi strain of vaccinia obtained from Dr. T. M. Rivers. The material employed in all the experiments was a testicular suspension of this virus. In the first experiment only one injection of 0.25 cc. of a 10^{-4} dilution was given. In the second experiment 0.25 cc. of the following dilutions was injected intradermally 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}. To minimize error the injections in all the animals were made from the same dilutions, the same syringe being employed for each.

India Ink.—This was used in a dilution of 1 part India ink to 2 parts Locke's solution. The injection of India ink was chosen for use in these experiments as we wished to compare our results with those obtained by Hoffman and Duran-Reynals (11). In the second experiment to avoid the possibility that repeated injections of the India ink in the same rabbit might affect the spread of subsequent injections only a few rabbits were used in the control period. It is interesting to note that this was an unnecessary precaution as no significant differences appeared in the control group between the animals which had had one injection and those which had had several injections. All the animals, however, showed a slight increase in the spread of India ink at the end of the experiment.

Measurement of Area.—The figures given in the various tables for the number of square millimeters covered by the vaccinia lesions and covered by the India ink are the product of their two diameters. More precise information might have been obtained by using a planimeter but it was found that this changed the results only slightly.

Wheal Disappearance Time.—This test, which consists in the injection of 0.2 cc. of physiological saline intradermally into the skin and noticing the time it takes for the disappearance of the wheal, has been used in studying certain intoxications. The literature concerning this test has been reviewed by Bradford (12). Although Hoffman and Duran-Reynals (11) report that the wheals produced by testicular extract disappear more quickly than those produced by saline, we have not found any studies giving exact data on the correlation of these two tests. A more detailed consideration of this factor will be made in a future paper. It is included in this paper merely in corroboration of the India ink experiment.

Necropsy.—At the conclusion of the experiment, all of the rabbits were necropsied and all the organs studied both in the gross and microscopically for any remaining testicular tissue and also for the presence of any extraneous disease. One rabbit was found which had received no endocrine injection but did have a caseous lesion of the lung. It is interesting to note that this animal which was excluded from the experiment resembled more the animals which had received the estrogenic hormone than it did the other control animals. Another rabbit from

which all the testicle had not been removed and which had received the estrogenic hormone showed a marked reduction in the spread of India ink and failed to show any lesions from the vaccinia. Two other rabbits died during the course of the experiment and were also excluded.

EXPERIMENTS

The experiments were designed to study the ability of the estrogenic and the gonadotropic hormones to modify the tissues of the host to virus infection and to ascertain the effect they had on the spread of India ink.

Experiment 1.—13 rabbits were used in this experiment. 5 were given the estrogenic hormone, 4 the gonadotropic hormone, and 4 were used as controls. They were vaccinated on one side and the India ink injected on the other. The vaccinia lesions showed no essential differences and hence are not shown. The area covered by the India ink after 1 hour is shown in Table I.

Experiment 2.—This experiment was designed to extend the findings of Experiment 1 in regard to both the spread of the India ink and the vaccinia infection. The objectives of the experiment were to determine whether the hormones actually decreased the spread of India ink or merely delayed it and to study the resistance to vaccinia infection by using several dilutions of the virus as described above.

24 rabbits were used in this experiment. 8 were given the estrogenic hormone, 8 the gonadotropic hormone, and 8 were kept as controls. The spread of the India ink was measured after 1 hour and again after 24 hours, the results being shown in Table II. It was thought that measurement of the spread from the moment of injection would be of interest. The amount of increased spread in 1 hour is given in Table III. The data on the disappearance time of the wheals and the effect on the vaccinia infection are given in Tables IV and V.

The rabbits were vaccinated, as described above, the day following the last injection of the hormones. The lesions were measured daily and the results tabulated in Table V. The rabbits were killed 14 days after vaccination.

Interpretation of Tables

Table I: The Spread of India Ink.—This table shows clearly that both the estrogenic and the gonadotropic hormones decrease the spread of India ink through the skin.

Table II: The Spread of India Ink.—This table shows that there is considerable variation in the spread of the India ink in the control animals. The variation, however, is about the same as that found by Hoffman and Duran-Reynals (11). If their figures are treated as ours were we find that in 11 controls the spread of the India ink

TABLE I

The Spread of India Ink

Rabbit No.	Estrogenic	Rabbit No.	Gonadotropic	Rabbit No.	Control
	sq. mm.		*sq. mm.*		*sq. mm.*
1	180	6	270	10	720
2	260	7	270	11	600
3	250	8	255	12	400
4	300	9	195	13	500
5	196				
Average....	237		247		555

TABLE II

The Spread of India Ink

Rabbit No.	Before injection		During injection Periods of time from first injection								14 days after stopping injections	
			7 days		14 days		21 days		23 days			
	1 hr.	24 hrs.	1 hr.	24 hrs.	1 hr.	24 hrs.	1 hr.	24 hrs.	1 hr.	24 hrs.	1 hr.	24 hrs.
	sq. mm.	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*
					Estrogenic							
14	418	625	320	800	266	580	300	360	289	225	460	460
17	400	400	228	560	380	440	270	—	300	330	475	408
20					280	667	300	255	225	460	441	391
23					238	550	257	361	361	289	400	418
26							255	520	300	520	432	500
29							285	380	266	380	480	575
32							288	316	306	504	483	442
Average			274	680	291	559	279	365	293	358	453	477
					Gonadotropic							
15	418	400	320	525	304	—	340	460	532	768	460	460
18	420	352	340	494	374	374	475	1600	550	837	506	—
21			432	374	374	875	425	810	414	851	440	340
24					374	405	320	925	418	928	675	399
27							385	320	357	360	550	512
30							456	506	300	560	440	—
33							352	546	336	616	546	546
Average			364	464	356	551	393	738	415	703	517	451
					Controls							
16	440	400	352	475	418	456	440	440	238	400	456	460
19	342	400	396	500	380	304	360	650	462	440	504	375
22			300	500	336	330	500	—	414	594	484	520
25					320	—	351	500	396	640	425	399
28							440	616	396	660	450	475
31							385	500	400	560	462	728
Average	406	430	349	492	363	363	413	541	384	549	464	493

varied from 285 to 575 sq. mm. with an average spread 516 sq. mm. In the table there are 31 control readings which show a variation

TABLE III

The Increased Area Covered by India Ink after 1 Hour

Rabbit No.	Before injection	During injection Periods of time from first injection				14 days after stopping injections
		7 days	14 days	21 days	23 days	
	sq. mm.	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*
			Estrogenic			
14	193	95	41	75	64	235
17	175	3	155	45	75	250
20			55	75	0	216
23			13	32	136	175
26				30	75	207
29				60	41	255
32				63	81	258
Average...		49	66	54	68	228
			Gonadotropic			
15	193	95	79	115	307	235
18	195	115	149	250	325	281
21		207	149	200	189	215
24			149	95	193	450
27				160	132	325
30				231	75	215
33				127	111	321
Average...		139	132	168	190	292
			Controls			
16	215	127	193	215	13	231
19	117	171	155	135	237	279
22		75	111	275	189	259
25			95	126	171	200
28				215	171	225
31				160	175	237
Average...	181	124	139	188	159	239

between 300 and 504 sq. mm. with an average of 413 sq. mm. Hoffman and Duran-Reynals (11) mention in their paper that they found no significant increase in the spread of the India ink after 1 hour. In

the table it will be observed that there is usually a slight increase in the spread of the India ink in the control animals but we do not believe that this is large enough to be considered significant.

TABLE IV

Wheal Disappearance Time

Rabbit No.	Before injection	During injection Periods of time from first injection				14 days after stopping injections
		7 days	14 days	21 days	23 days	
	min.	*min.*	*min.*	*min.*	*min.*	*min.*
			Estrogenic			
14	75	45	90	90	120	75
17	75		45	90	75	60
20			75	90	165	60
23			90	75	135	135
26				120	165	60
29				150	150	75
32				135	90	90
Average...			75	107	129	79
			Gonadotropic			
15	60	45	90	45	105	120
18	75		60	60	105	90
21			45	60	75	75
24			45	90	90	105
27				75	60	45
30				45	45	75
33				45	75	60
Average...			60	60	79	81
			Controls			
16	60	45	45	45	60	75
19	45		60	45	60	45
22			45	45	90	90
25			45	45	75	60
28				45	75	45
31				75	90	60
Average...	65	45	49	50	75	63

The animals which received the estrogenic hormone showed a decrease in the 1 hour spread of the India ink after the hormones had

been given for only 1 week. The spread of India ink after 24 hours was not only not decreased during the first 2 weeks but showed a

TABLE V

Vaccinia Lesions

Rabbit No.	Titration of virus	Maximum size of lesions				Maximum size of lesions at end of experiment
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	
		sq. mm.	*sq. mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*
		Estrogenic				
14	100×10^{-7}	320	49	0	0	0
17	100×10^{-7}	100	36	0	0	4
20	1000×10^{-7}	240	0	0	0	9
23	100×10^{-7}	400	256	0	0	121
26	100×10^{-7}	400	144	0	0	36
29	100×10^{-7}	360	324	0	0	165
32	100×10^{-7}	625	225	0	0	150
Average...	229×10^{-7}	349	172			69
		Gonadotropic				
15	10×10^{-7}	270	180	121	0	25
18	10×10^{-7}	352	272	288	0	100
21	10×10^{-7}	360	121	81	0	100
24	10×10^{-7}	225	400	225	0	144
27	100×10^{-7}	594	225	0	0	550
30	1×10^{-7}	400	144	25	9	36
33	1×10^{-7}	324	81	0	81	64
Average...	20×10^{-7}	361	203	106	13	145
		Control				
16	10×10^{-7}	550	196	9	0	120
19	1×10^{-7}	625	144	25	4	625
22	10×10^{-7}	256	84	100	0	81
25	1×10^{-7}	500	144	144	144	500
28	100×10^{-7}	340	100	0	0	16
31	100×10^{-7}	308	100	0	0	0
Average...	37×10^{-7}	430	128	46	25	224

slight increase over the controls. After 3 weeks the spread of India ink was decreased after 24 hours as well as 1 hour.

It should be observed in Table II that in the 1 hour readings of 31

controls there is only 1 instance below 300 sq. mm., whereas the 20 one hour readings while the estrogenic hormone was being given show only 4 instances above 300. The same applies to the 24 hour readings of those which had received the estrogenic hormones for 3 weeks, there being only 3 above 400 sq. mm. in this group and in the control group only 4 below 400 sq. mm.

It will be seen both in Table II and Table III that the gonadotropic hormone did not cause a decrease in the spread of India ink as it did in Experiment 1.

Table III: The Increased Area Covered by India Ink after 1 Hour.—This table shows the increase in size of the area covered after 1 hour rather than the total area. This is done to bring out more clearly the effect of the estrogenic hormone in limiting the spread of the ink.

Table IV: Wheal Disappearance Time.—The results of this experiment run parallel to the India ink one in that the longer the wheal persisted the less was the spread of the India ink. The disappearance time of the wheals had returned to normal in every case 2 weeks after discontinuing the injection of the hormones.

Table V: Vaccinia Lesions.—This table shows that the rabbit's resistance to infection with vaccinia is increased if the rabbit has been given large amounts of the estrogenic hormone before it is vaccinated. There does not, however, appear to be any great difference in the maximum size of the lesion if the virus was able to take hold. It also shows that if the virus is of sufficient strength to cause a lesion in the animal then this lesion will in most instances reach the same size as the ones in the control group. This is in agreement with Experiment 1. The average size of the lesions at the end of the experiment shows that the animals given the estrogenic hormone recovered from their skin lesions more quickly than the control animals. However, since there is such wide variation in size we prefer to wait until we have further data before stressing this point.

DISCUSSION

At present it does not seem advisable to attempt to explain how these changes occur except that they apparently are related to the permeability of the tissue to both fluids and particulate matter. It does appear, however, that there is present in the estrogenic hormone

some factor or factors that have an effect on the tissues of the body opposite to that of simple extracts of the testis. It is thought that further study of this factor or factors will lead to a better understanding of the modifications which have been seen to occur as a result of pregnancy in tumor growth, infection, and allergic states. It also gives us a new method of studying the reactions of the host to numerous incitants of disease.

CONCLUSIONS

The essential findings of these experiments may be summarized as follows:

The estrogenic hormone after being given for 1 week slows up the spread of India ink but allows it to reach and exceed a normal spread after 24 hours.

After injections of the estrogenic hormone for 3 weeks the spread of India ink is much less than in the control animals.

The resistance of the rabbit to vaccinia is increased if the rabbit has been castrated and then given the estrogenic hormone for a period of 3 weeks before being vaccinated.

At the present time nothing can be said about the action of the gonadotropic hormone on India ink, as the experiments did not agree.

BIBLIOGRAPHY

1. Sprunt, D. H., *J. Exp. Med.*, 1932, **56**, 601.
2. Bainbridge, W. S., *Am. J. Obst. and Dis. Women and Child.*, 1918, **77**, 21.
3. Williams, J. W., Obstetrics, New York, D. Appleton & Co., 5th edition, 1926, 546.
4. Rosahn, P. D., Hu, C. K., and Pearce, L., *J. Immunol.*, 1936, **31**, 59.
5. Aycock, W. L., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 573.
6. Kemp, J. E., *J. Infect. Dis.*, 1937, **60**, 32.
7. Williamson, A. C., *Am. J. Obst. and Gynec.*, 1930, **20**, 192.
8. Manwaring, W. H., in Jordan, E. O., and Falk, I. S., The newer knowledge of bacteriology and immunology, Chicago, The University of Chicago Press, 1928, 989.
9. Solomonica, B., and Kurzrok, R., *Endocrinology*, 1936, **20**, 171.
10. Bray, G. W., Recent advances in allergy, Philadelphia, P. Blakiston's Son & Co., 3rd edition, 1937, 107.
11. Hoffman, D. C., and Duran-Reynals, F., *J. Exp. Med.*, 1931, **53**, 387.
12. Bradford, W. L., *Am. J. Dis. Child.*, 1927, **34**, 950.