normally found in the estrus-ovulatory period, leads me to believe that after activation of the rabbit's own pituitary, the mechanism of ovulation is probably the same as after coitus.

Summary. The response to pregnancy urine of the rabbit anterior pituitary is one of increased oxygen consumption in the 7-12-hour interval after injection. The inference may be drawn that the effect of pregnancy urine is to activate the anterior pituitary leading to the output of an effective dose of rabbit hormone after an interval of 12-24 hours.

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The Stage of Mitosis at which Chromosomes are Rendered Less Sensitive to X-rays by Ammonia.

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On the basis of data obtained in previous experiments, it has been postulated that the marked sensitivity to X-rays of chromosomes at the onset of the prophase may be attributed to the neutralization by negative electrons of positive charges carried by the adjacent surfaces formed upon division of the chromonemata.[1] Later experiments showed that in keeping with this hypothesis, treatment with CO_2 did not alter the reaction of chromosomes to X-rays, while NH_4OH greatly reduced the number of chromosome abnormalities produced by a given dose of X-rays.[2] The theory further required that in normal mitosis the positive charges appear at the onset of the prophase and then disappear during the course of the prophase, presumably as a result of the combination of the positively charged groups with nucleic acids. In accordance with this hypothesis a penetrating base such as NH_4OH would be expected to reduce the X-ray sensitivity of chromosomes primarily at the onset of the prophase. The experiments to be described were designed to determine whether these expectations would be fulfilled.

Onion seedlings* were irradiated with X-rays and at various intervals after irradiation the root tips examined for the percent anaphases which had no chromosome abnormalities. Similar seed-

1 Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

2 Marshak, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 705.

* Sweet Spanish, Riverside Strain, Ferry-Morse Seed Company.

lings were immersed in 0.0025 N NH_4OH for 30 minutes and then irradiated. The technic used has already been described.[2] Both groups of seedlings were given 80 roentgens of X-rays. The source of radiation was an oil-cooled X-ray tube† with a tungsten target which was operated at 200 K.V. and 25 ma. with 2 valve rectification. The filtration by the glass, oil and bakelite was equivalent to 2 mm Al. and 0.25 mm Cu. The effective wave-length was 0.235Å. The distance from target to seedlings was 80 cm and the time for irradiation 112 seconds. By accident one lot of seedlings received only 75 r.

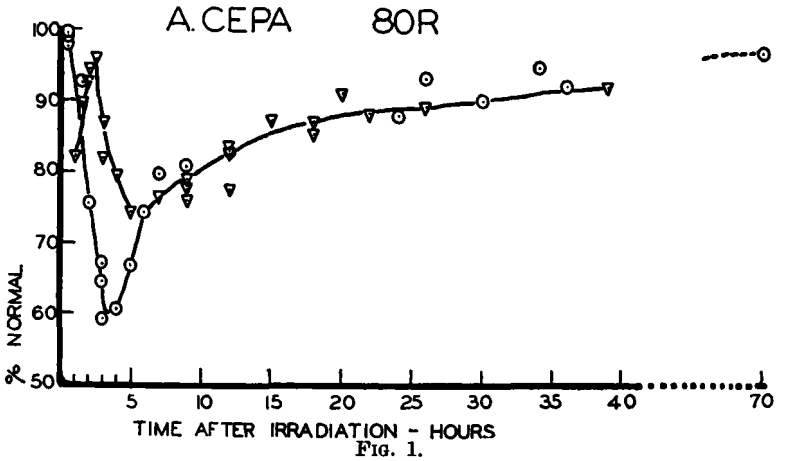

FIG. 1.

Ordinates, % normal anaphases. Abscissæ, time in hours after irradiation. Circles represent data from seedlings treated only with X-rays, the triangles seedlings treated with ammonia and X-rays. With the exception of 3 points all were obtained from seedlings given 80 roentgens. The 3 exceptions are the topmost circle at 3 hours and the circles at 7 and 9 hours which by accident received only 75 roentgens.

The results obtained are shown in Fig. 1, where the percent normal anaphases are plotted as a function of time after irradiation. The data upon which these curves are based are shown in Tables I and II. Counts of 700 anaphases in untreated control seedlings showed 0.7% abnormal with respect to chromosome separation. As has been previously described for this and other species, there is a pronounced minimum in the percent normal anaphases at 3 hours for cells treated only with X-rays. In marked contrast, the seedlings treated with ammonia and X-rays showed a sharp maximum in the percent normal anaphases at 2.5 hours after irradiation or 3 hours

† Purchased from the Picker X-ray Corporation. Style 1935, Serial No. 109, tube No. 10264.

TABLE I.
X-ray—Time Curve—Experiments No. 64 = 80 r; 171 = 80 r; 177 = 75 r.
A. cepa.

Time after X-ray in hr	Exp. No.	Slide No.	n	a	Total	% n
0.5	64	517	350	9	359	97.5
	171	1555	565	4	569	99.5
	64	526	396	3	399	99.4
1.0						
1.5	64	527	266	22	288	92.4
2.0	171	1556	163	52	215	75.8
3.0	177	1577	248	122	370	67.0
	171	1557	222	154	376	59.1
	64	528	148	81	229	64.5
4.0	171	1558	206	135	341	60.4
5.0	171	1559	238	118	356	66.9
6.0	64	529	159	56	215	74.2
7.0	177	1578	445	113	558	79.9
9.0	177	1579	345	81	426	81.0
12.0	177	1580	601	125	726	82.9
24.0	64	530	278	37	315	88.3
26.0	171	1560	610	42	652	93.5
30.0	64	532	360	39	399	90.3
34.0	64	535	757	40	797	95.0
36.0	64	523	649	54	703	92.4
70.0	64	536	701	20	721	97.2

after the beginning of the ammonia treatment. By 5-6 hours the curves for the two groups are identical.‡

It has been shown previously that the cells from which the minimum percent abnormal anaphases are derived at 3 hours after irradiation were in the onset of the prophase during the exposure to the X-rays. Accordingly the cells seen in anaphase at 5 to about 25 hours after irradiation were in various phases of development in the resting stage when exposed. One may conclude, therefore, that ammonia does not alter the sensitivity to X-rays of chromosomes in the resting stage.

The points at 1-2 hours after irradiation represent counts of cells

‡ The points obtained from the lot of seedlings which received only 75 r are represented in Fig. 1 by the topmost circle at 3 hours and the circles at 7 and 9 hours. As was to be expected they were somewhat higher than the curve for 80 r but are considered sufficiently close to be included in this group.

TABLE II.
NH_4OH + X-ray—Experiments Nos. 164, 165, 176, 178, 179, 181.
A. cepa.

Time after X-ray in hr	Exp. No.	Slide No.	n	a	Total	% n
0.5						
1.0	164	1526	481	108	589	82.0
1.5	178	1583	462	52	514	89.9
2.0	178	1584	168	14	182	92.4
	164	1529	341	24	365	94.5
2.5	178	1585	71	3	74	96.0
3.0	176	1569	153	34	187	82.0
3.0	164	1527	387	58	445	87.0
4.0	164	1530	301	78	379	79.5
5.0	176	1570	208	72	280	74.4
7.0	176	1571	279	87	366	76.3
9.0	179	1595	114	33	147	77.6
	176	1572	237	75	312	76.0
	181	1603	75	20	95	79.0
12.0	178	1586	454	89	543	83.6
	176	1573	439	126	565	77.6
	178	1587	615	126	741	83.0
15.0	178	1588	823	118	941	87.4
18.0	178	1589	744	110	854	87.1
	178	1590	434	73	507	85.5
20.0	178	1591	718	68	786	91.3
22.0	176	1574	316	41	357	88.6
26.0	176	1575	829	98	927	89.5
39.0	178	1592	726	63	789	92.1

which were in the prophase when irradiated. The percent normal anaphases in the cells receiving ammonia and X-rays at 1 and 2 hours after irradiation are approximately the same as for cells treated only with ammonia (Table IV in [2]). For the seedlings receiving ammonia and X-rays the values are 82.0 and 93.0% respectively and for those receiving only ammonia they are 87.0 and 92.4, These results show that ammonia completely protects the chromosomes from the action of the X-rays during the earlier prophases, and in accordance with the hypothesis indicate the presence of some positive charges in the chromonemata during these stages. In Fig. 1 it can be seen that at 1.5 hours after irradiation there is little difference between cells treated with X-rays alone and those receiving ammonia and X-rays. By 2 hours after irradiation there is a wide divergence between the values of the 2 groups indicating that in the

normal mitosis the positive charges disappear quite rapidly during the early prophase.

Irradiation with X-rays not only produces chromosome abnormalities, but reduces the number of cells entering the prophase. The data obtained show a marked reduction in the number of cells in anaphase with time, which reaches a minimum at about 3 hours after irradiation.[1] If the ammonia is considered to act on the nuclei within a few minutes after the seedlings are immersed, the maximum in the percent normal anaphases comes at 3 hours after the action of the ammonia. Ammonia also reduces the number of cells in anaphase from about 1,100 per 30 roots at 0.5 hour after immersion to a minimum of 370 at 3 hours after irradiation (Table IV). This is taken to indicate that ammonia not only suppresses the positive charges on the dividing chromonemata, but also inhibits the onset of the prophase. However, in sharp contradistinction to X-rays ammonia produces the most chromosome abnormalities in the metaphase when the X-ray action is so slight as to be hardly or not at all detectible.

These results, therefore, support the hypothesis that positively charged surfaces are developed in the chromonemata at the onset of the prophase and are gradually neutralized during the prophase. They also suggest that ammonia, like X-rays, inhibits cells from entering the prophase.

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Changes in Protein-Content of Bacterial Suspensions During Lysis and Autolysis.

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The lysis of bacteria by phage has been considered as a simple plasmolysis, quite different from autolysis, and in fact many attempts to detect protein split products in the solution after lysis have failed. Bronfenbrenner and Hetler,[1] however, did find evidence for the appearance of protein split-products in solution after lysis and hence concluded that lysis was due to hydrolysis of the protein of the bacterial cell.

[1] Bronfenbrenner, J., and Hetler, D. M., *J. Exp. Med.*, 1928, **48**, 269.