mice, guinea pigs, and other animals which had been infected or immunized, it is believed that the fixation-reactions of the sera of virus-free parrots are indicative of a past infection. It must be reserved for future studies to decide if these reactions are also indicative of immunity. The complement-fixation test in its present state does not distinguish an infection from a sterile immunity. From the standpoint of public health, parrots which specifically react in the complement-fixation must be destroyed.

## 10611

## Effects of Fast Neutrons on Chromosomes in Mitosis.*

A. MARSHAK.† (Introduced by John H. Lawrence.)

*From the Radiation Laboratory, University of California, Berkeley, Calif.*

The neutron source was a beryllium target bombarded by deuterons accelerated to 8 million volts in the cyclotron of Lawrence and Cooksey.[1] The neutrons so obtained were collimated in a beam as described by Aebersold.[2] The biological material to be treated was placed just outside the 10 x 10 cm port at the end of the collimation apparatus at a distance of 70 cm from the target. Ionization produced by the neutrons was measured in arbitrary "n" units, and "n" unit being that amount of ionization produced by neutrons which gives the same reading on a 100 r Victoreen thimble ionization chamber as does one roentgen of X-rays.

Six-day-old seedlings of *Vicia faba* and *Pisum sativum* were mounted on an annular wooden holder and oriented so that the root tips lay in the center of the 3-inch aperture which was covered on either side by a sheet of wet filter paper and a sheet of celluloid 5.4 thousandths of an inch thick. The cotyledons and epicotyl of the seedlings lay outside the neutron beam, so that corrections for scattering may be neglected. The much smaller seedlings of *Solanum lycopersicum* were mounted between 4 sheets of wet filter paper 3½ inches in diameter lying between 2 sheets of cellophane held on a round wooden embroidery hoop 6 inches in diameter. All seedlings

* Supported by a grant from the Committee on Radiation of the National Research Council.

† Fellow of the John Simon Guggenheim Memorial Foundation.

1 Lawrence, E. O., and Cooksey, D., *Phys. Rev.*, 1936, **50**, 1131.

2 Aebersold, P. C., *Phys. Rev.*, 1939, in press.

were kept at 23°C before and after treatment. The mouse tumors were treated *in vivo* by placing the animal which was tied on a piece of cardboard so that the tumor was in the center of the neutron beam. To obtain the dose given the tumor an ionization chamber was left immediately behind the tumor during exposure. For examination of the chromosomes only the tumor tissue adjacent to the ionization chamber was used. Exposure times varied from 5 to 30 minutes.

The root tips and tumor tissue were fixed at various intervals after the neutron treatment. From these, smear preparations stained with acetocarmine were made and examined with the microscope. In *V. faba* there were 6 instances in all the anaphases examined (approximately 5,000) in which a section of a chromosome was spread out and markedly disorganized. Such chromosome disorganizations have not been observed with X-rays. Otherwise, the chromosome abnormalities observed in anaphase were qualitatively the same as those previously described for X-rays.

As with X-rays the percent normal anaphases (those showing no chromosome abnormalities) decreases to a minimum at 3 to 9 hours after irradiation and then increases again.[3] An example is given in Fig. 1, where the percent normal anaphases is plotted as a function of time after treatment of roots of *V. faba* with 20 "n". Similar curves were obtained for all the plants used after treatment with various doses of neutrons. When the logarithm of the minimum values of the time curves are plotted as a function of dose in "n" units, straight lines are obtained as shown in Fig. 2. The data from which these curves were obtained are given in Table I.

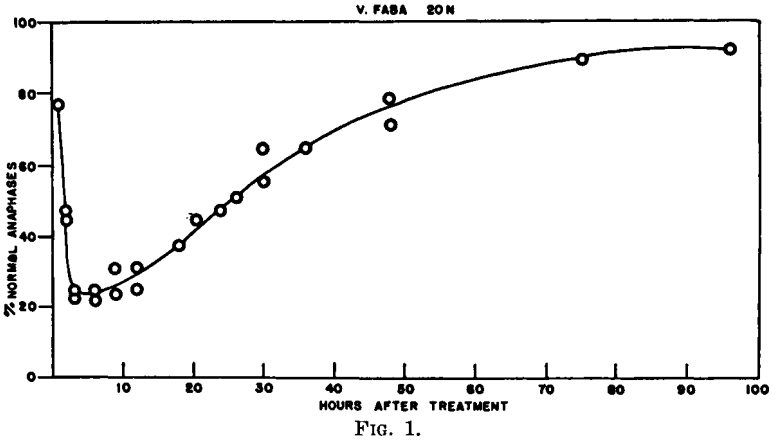


FIG. 1.

Percent normal anaphases in *Vicia faba* root tips as a function of time after treatment with 20 "n" units of neutrons.

[3] Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

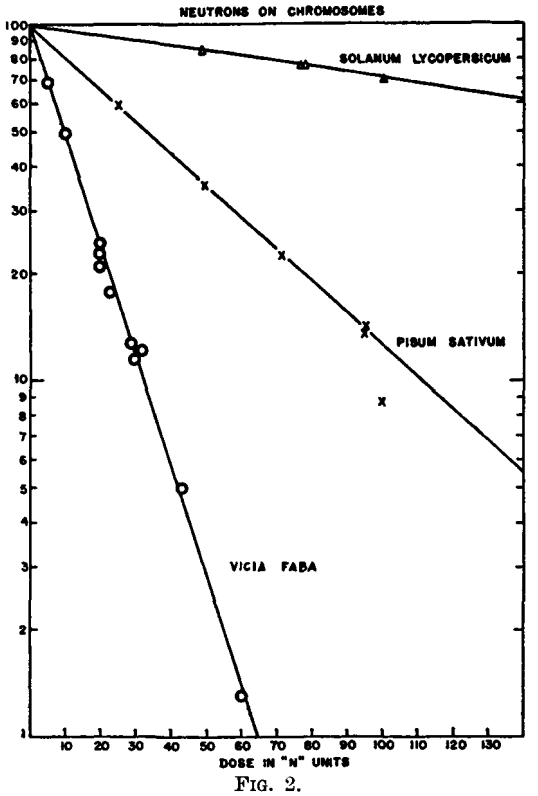


FIG. 2.

Percent normal anaphases as a function of dose of neutrons in "n" units. Ordinates percent normal anaphases at the minima of the time curves plotted on a logarithmic scale. Abscissæ, dose in "n" units. Circles represent *V. faba*, crosses *P. sativum*, triangles *S. lycopersicum*.

Three types of mouse tumors were each given 30 "n". The percent normal anaphases at 3 hours after irradiation are given in Table II.

The differences in percent normal anaphases for the 3 tumors are not considered significant in view of the possible differences in actual dose received by the tissue examined. These tumors show marked differences in sensitivity to X-rays as determined by tumor regression *in vivo* or survival of tumor fragments irradiated *in vitro*. This suggests that factors other than chromosomes may be responsible for these differences or that the loss of chromosome fragments in some types of tumors is more likely to have a lethal effect than in others.

The slopes of the survival curves of the plants and the data from the mouse tumors may be compared with the survival curves previously obtained for the chromosomes of these organisms with X-rays, as shown in Table III.

The factor of *6* obtained for the ratio of the neutron to X-ray

TABLE I.
Cells with Chromosome Abnormalities in Anaphase Three Hours After Neutron Treatment.

| Dose in "n" units | Normal cells | Abnormal cells | *V. faba* Total | *V. faba* % normal |
|---|---|---|---|---|
| 5 | 177 | 78 | 255 | 69.4 |
| 5 | 985 | 396 | 1381 | 71.2 |
| 10 | 189 | 192 | 381 | 49.6 |
| 10 | 473 | 479 | 952 | 49.7 |
| 20 | 25 | 96 | 121 | 20.6 |
| 20 | 122 | 401 | 523 | 23.4 |
| 20 | 138 | 435 | 573 | 24.2 |
| 23 | 64 | 290 | 354 | 18.0 |
| 23 | 17 | 78 | 95 | 17.9 |
| 29 | 31 | 215 | 246 | 12.6 |
| 30 | 21 | 161 | 182 | 11.5 |
| 32 | 16 | 114 | 130 | 12.3 |
| 43 | 2 | 37 | 39 | 5.1 |
| 60 | 2 | 152 | 154 | 1.3 |
| 0 | 1214 | 14 | 1228 | 99.0 |
| | | | *P. sativum* | |
| 25 | 539 | 364 | 903 | 59.7 |
| 50 | 172 | 314 | 486 | 35.4 |
| 71 | 70 | 239 | 309 | 22.6 |
| 95 | 32 | 242 | 274 | 14.4 |
| 95 | 41 | 255 | 296 | 13.9 |
| 100 | 12 | 124 | 136 | 8.8 |
| 0 | 1398 | 12 | 1410 | 99.0 |
| | | | *S. lycopersicum* | |
| 49 | 163 | 29 | 192 | 85.0 |
| 77 | 69 | 23 | 92 | 75.0 |
| 78 | 35 | 11 | 46 | 76.1 |
| 100 | 141 | 62 | 203 | 69.5 |
| 0 | 243 | 3 | 246 | 98.8 |

slope in all the species studied supports the hypothesis previously advanced on the basis of the X-ray data alone, that the cross-sectional area of the portion of the chromonema which is sensitive to the ionization is approximately the same size (same order of magnitude) in all of them.§ The separation of ion pairs along the track of a $\beta$

TABLE II.

| Type Tumor | Normal Anaphases | Abnormal Anaphases | Total | % Normal |
|---|---|---|---|---|
| Sarcoma 180 | 417 | 82 | 499 | 83.6 |
| Mammary carcinoma | 304 | 53 | 357 | 85.1 |
| Lymphosarcoma | 1100 | 168 | 1268 | 86.9 |

§ In this connection it should be noted that neutron-X-ray ratios varying from 2 to 5 were observed when the survival of wheat seedlings, *Drosophila* eggs, fern spores, mice and mouse tumors was investigated.[4,5,6]

[4] Zirkle, R. E., Aebersold, P. C., and Dempster, E. R., *Am. J. Cancer*, 1937, **29**, 556.

[5] Zirkle, R. E., *Occasional Publications of the A.A.A.S.*, 1937, **4**, 220.

[6] Lawrence, J. H., Aebersold, P. C., and Lawrence, E. O., *Occasional Publications of the A.A.A.S.*, 1937, **4**, 215.

TABLE III.
Slopes of Survival Curves.

| Species | Neutrons | X-ray | Neutron-X-ray |
|---|---|---|---|
| *V. faba* | 70.4 x 10–3 | 10.7 x 10–3 | 6.6 |
| *P. sativum* | 20.5 x 10–3 | 3.3 x 10–3‡ | 6.2 |
| *M. musculus* | 5.4 x 10–3 | 9.3 x 10–4 | 5.8 |
| *S. lycopersicum* | 3.5 x 10–3 | 5.4 x 10–4 | 6.5 |

‡ Due to an error this slope was previously given as 4.3 x 10–3. [3]

particle from X-rays is of the order of $10^{-5}$ cm. With neutrons most of the ionization in tissue is along proton tracks where the separation of ion pairs is of the order of $10^{-7}$ cm. If there were marked differences in the cross section of the sensitive portion of the chromonema a considerable variation in the neutron-X-ray ratio was to be expected.

From the X-ray data the diameter of the sensitive portion of the chromonema was calculated to be about $10^{-7}$ cm on the assumption that the ion pair was the agent effective in producing chromosome abnormalities. On the same assumption neutron ionization would be expected to be less efficient than X-rays in producing abnormalities if the sensitive diameter were much greater than the average separation of ions along proton tracks. Similarly if clusters of ions rather than pairs were necessary in order to produce abnormalities, neutron ionization should be more efficient. If the sensitive structure is of the same order of magnitude as the average distance between ion pairs of the proton track or smaller, little difference in efficiency between X-ray and neutron ionization is to be expected.

The factor 6 obtained for the neutron-X-ray ratio may be due to a greater efficiency of the neutron ionization. A similar result might be obtained if the "n" unit were 6 times larger than the roentgen. However, experiments on the physical measurement of neutron ionization conducted in this laboratory indicate that the "n" unit is not larger than the roentgen by a factor greater than 2.5 (Aebersold and Anslow, unpublished). If these measurements be accepted, the neutron ionization is about 2.5 times as efficient as the ionization produced by X-rays. There seems to be no obvious explanation for such a factor. Therefore, before reaching any definite conclusions as to whether ion pairs or ion clusters are the effective agents in producing chromosome abnormalities, it seems desirable to make a determination of the efficiency of protons which would not be dependent upon ionization chamber measurements. Such experiments are now being undertaken.