valents a few days after treatment which they attribute to asynapsis or desynapsis. On the other hand, Darlington[4] reports that colchicine induces crossing over in regions where it is normally excluded. Further data on the influence of colchicine on meiotic chromosome pairing is presented in the present paper and the results treated statistically.

Young potted cuttings from a white-flowered clone of *Antirrhinum majus* L. (2n = 16) were treated by immersion in aqueous solutions of colchicine (0.1, 0.15 and 0.25 per cent.) for periods ranging from seven to 42 hours. After treatment the plants were grown in the greenhouse until flower buds were sufficiently developed to obtain pollen mother cells. The time elapsed between treatment and fixation varied from 6 to 15 weeks. Control material was collected from untreated plants of approximately the same age, most of which had been immersed in water while the treated plants were in the colchicine bath.

The number of lagging univalents at first anaphase were scored for one hundred or more cells from each of 30 control and 52 treated branches. The number of cells examined and the percentage of cells with 0, 1, 2, 3 and 4 laggards are given in Table 1. In the control plants 2.61 per cent. of the cells had one or more laggards, while the treated plants had 3.58 per cent. This is an increase of about 37 per cent. The χ^2 test shows that the probability of a difference of this magnitude being due to chance alone is less than one per cent. (Table 1) or if all the cells with laggards are grouped together the fourfold table test of goodness of fit again shows the probability to be less than one per cent. The increase in number of univalents can, therefore, be regarded as highly significant.

TABLE 1

PERCENTAGES OF CELLS WITH 0, 1, 2, 3 AND 4 UNIVALENTS IN CONTROL AND COLCHICINE TREATED DIPLOID ANTIRRHINUM MAJUS L.

		Univalents					Total number of cells
		0	1	2	3	4	
Controls	Actual	3765	48	52	0	1	3,866
	Percentage	97.387	1.242	1.345	0	0.026	100
Treated	Actual	6596	96	144	4	1	6,841
	Percentage	96.419	1.403	2.105	0.058	0.015	100
$\Sigma\chi^2 = 9.324$		df* = 2		P = < 0.01			

*There are only two degrees of freedom because the numbers of cells with 3 and 4 univalents are too small to be considered separately and must be added to the two univalent class.

Since the univalents probably result from a decrease in number of chiasmata it can be concluded that colchicine must reduce crossing over in at least one pair of chromosomes. In the majority of the material examined meiosis did not occur until eight weeks or longer after the treatment. This would indicate either that colchicine or colchicine derivatives must remain in the plant for a considerable length of time, or that the treatment alters the structure of the chromosomes to such an extent that normal crossing over and chiasma formation is inhibited in a small percentage of cases. Complete inhibition of all crossing-over has been reported in pollen mother cells examined a few days after treatment. So far as the author is aware no previous reference has been made to such a long-term effect of colchicine on chromosome behavior.[5]

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CRYSTALLIZATION OF A PROTEIN FROM POLIOMYELITIS INFECTED MOUSE BRAIN[1]

A FRACTION which is essentially protein in nature has been obtained from the brains of mice infected with poliomyelitis virus. This fraction is birefringent, and the washed material (crystalline or liquid crystalline) is infective, producing typical symptoms of poliomyelitis. It was obtained by the following procedure: poliomyelitis infected brains were frozen and kept in a box with dry ice. Throughout the procedure the temperature was maintained at or below 0° and all manipulations were carried out under sterile precautions. Groups of between 10 and 15 brains were thawed and then extracted twice with saline 1:10 for one hour at pH 7.8. After centrifugation for 30 minutes at 2,500 R.P.M., the supernatant fluid was shaken with an equal volume of ether, which was added in small portions to the brain extract in a separatory funnel. Complete separation usually occurs after 6 to 8 hours in the refrigerator. The lowest layer in the separating funnel is only slightly opalescent and contains most of the virus.[2, 3, 4] From this layer, after separation, ether was removed by negative pressure. The solution was adjusted to pH 4.0 with N acetic acid and centrifuged. The supernatant (I) was kept separate. The precipitate was resuspended in saline, the pH adjusted to 8.0, thoroughly mixed with a glass rod and again centrifuged (supernatant

[4] C. D. Darlington, John Innes Hort. Inst. Ann. Report for the year 1940.

[5] This work was largely done under Bankhead-Jones Project Nos. 3 and 4 at the New York State Agricultural Experiment Station, Geneva, N. Y.

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] B. Howitt, *Proc. Soc. Exp. Biol. Med.*, 28: 158, 1930.

[3] M. Schaeffer and W. Brebner, *Archives Path.*, 15: 221, 1933.

[4] P. F. Clark, A. F. Rasmussen and W. C. White, *Jour. Bact.*, 42: 63, 1941.

II). Supernatant I and II were mixed and kept in a dry ice-box. The extracts obtained by the above procedure from several groups of infected brains were combined. This mixture was precipitated with 1.6 M ammonium sulfate at pH 7.0. The mixture was centrifuged and the precipitate discarded. The supernatant was then reprecipitated at pH 5.6 with 2.3 M ammonium sulfate and left for two hours in the refrigerator. The centrifuged precipitate was suspended in physiological saline and dialyzed in a Cellophane tube against saline for three days. The saline was changed every few hours. The dialyzed solution was brought to a pH of about 4.3 with n/10 acetic acid and the centrifuged precipitate discarded. To the clear, colorless supernatant n/100 acetic acid was very carefully added, drop by drop, until a first precipitate appeared. This precipitate was examined under a polarizing microscope and was found to consist partly of birefringent matter. One of these conglomerates was separated, washed in n/1000 acetic acid and dissolved in a small amount of dilute NaOH. It dissolved with difficulty. It proved highly infective for mice, producing typical paralytic symptoms of poliomyelitis after intracerebral inoculation in 14 to 72 hours. Another group of mice treated with omission of the ether extraction and with slight modifications of the above-mentioned method gave a somewhat better yield of the crystalline material. The data on this latter method are, however, still incomplete.

Another conglomerate was separated under the polarizing microscope and an x-ray diffraction photo of the wet material was taken by Dr. Fankuchen. It showed, in addition to some undifferentiated low angle scattering, a distinct though diffuse halo at an angle corresponding to about 4.5 Å. A halo of this character seems to be characteristic of protein material.[5, 6]

As encouraging as these data are it must be stressed that there is no evidence and no claim that the crystalline material obtained by this procedure represents the poliomyelitis virus. The possibility that the virus is adsorbed on the protein can not be excluded.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ON A NEW PROTEASE FROM PILEUS MEXICANUS[1]

Pileus mexicanus is an arboreous plant about eight meters high, belonging to the family of the Caricaceae; it grows wild in different states of Mexico (Morelos, Guerrero, Colima, Campeche y Yucatan), and is commonly known as "cuaguayote." From fruits and leaves a latex is obtained that has great activity, similar to that of papaya. The latex, collected by making longitudinal incisions on the fruits, clots rapidly, becoming brownish yellow in color.

After drying the latex in vacuum at 45° C it becomes easy to pulverize, yielding a white powder, similar to pulverized papain.

The enzymatic activity was determined by the milk-clotting method (Balls and Hoover)[2] and by titulation with alcoholic KOH (Willstätter, Waldschmidt-Leitz, modified by Balls).[3] Table I shows the activity obtained.

TABLE I

Mg of enzyme	Activator	pH	Clotting time in seconds	Milk-clotting units	cc of alcoholic KOH
1	None	4.6	60	1.00	....
1	Cystein 0.05 M.	4.6	50	1.20	....
5	None	4.7	..	....	1.10
5	H_2S	4.7	..	....	1.35

The corresponding values obtained by Balls for raw papain[4] in milk-clotting units are 1.11 after activation with Na CN and 1.09 non-activated per mg of latex. By titulation with alcoholic KOH, the maximum activity obtained by Balls on 5 mg of raw papain is 1.00 cc after activation with H_2S. These values show that this enzymatic preparation has a slightly superior activity to papain.

Similarly to other enzymes of the papain type, it is activated by HCN, H_2S and cystein, and rendered inactive by H_2O_2 and I_2. However, the papain clots the citrated blood, while the protease from Pileus does not. Its antihelminthic power was tried on *Ascaris lumbricoides, Macracanthorhynchus hirudinaceus, Oxyurus equi* and an Ankylostomid, being strongly positive in all cases.

The control specimens, in boiled enzyme, remained alive eight hours after the experiment was begun.

[1] Syn. *Jacaratia mexicana* (Sessé et Moc. ex D.C.); *Pileus heptaphyllus* (Sessé et Moc.), Ramirez; *Leucopremna mexicana* (Sessé et Moc.), Stanley.

[2] A. K. Balls and S. R. Hoover, *Jour. Biol. Chem.*, 121: 737, 1937.

[3] A. K. Balls, T. L. Swenson and L. S. Stuart, *Jour. Assoc. Off. Agr. Chem.*, 18: 140–146, 1935.

[4] A. K. Balls, H. Lineweaver and S. Schwimmer, *Indust. and Eng. Chem.*, 32: 1277, 1940.

[5] J. D. Bernal, I. Fankuchen and M. Perutz, *Nature*, 141: 523, 1938.

[6] I. Fankuchen, *Annals New York Acad. Sciences*, 41: 157, 1941.

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