

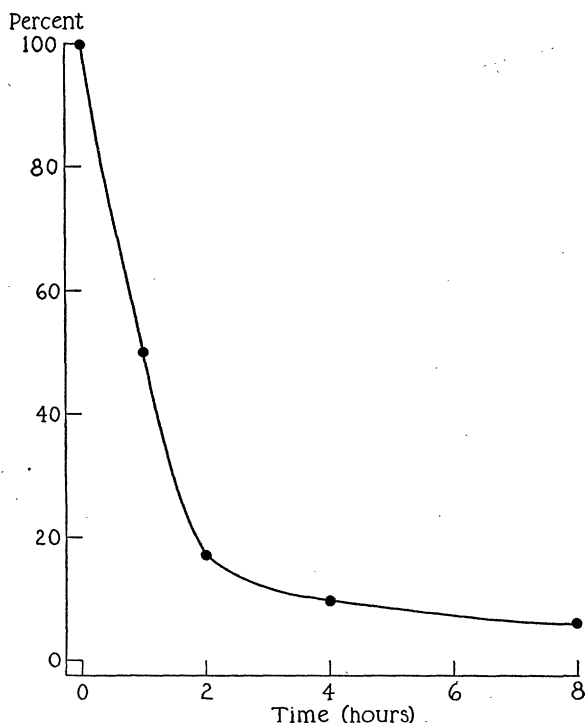


FIG. 1. Per cent. radioactivity remaining at site of injection.

In Fig. 1 the percentage of radioactivity remaining at the site of injection is plotted against time.

In Fig. 2 is shown the relation between the rate of

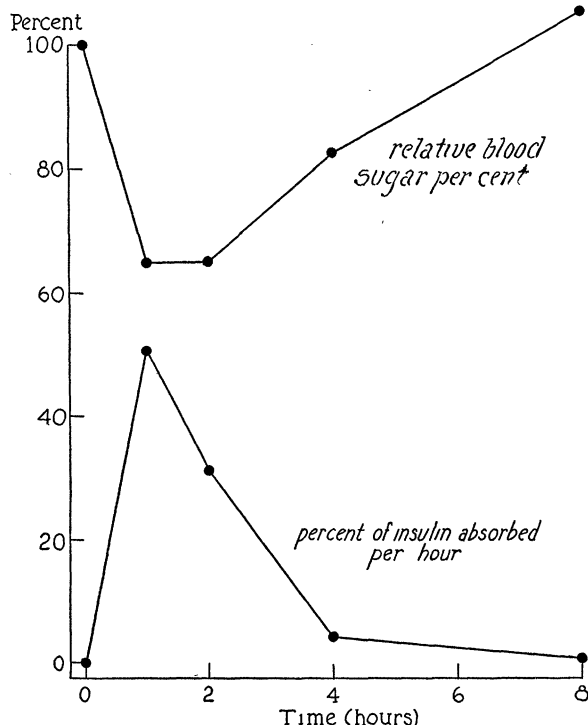


FIG. 2. Comparison of the rate of absorption of radioactive insulin with blood sugar levels.

absorption of radioactive insulin (*i.e.*, the percentage of radioactive material absorbed per hour since the last measurement) and the blood sugar at various times following the injection. From this graph it appears that the time of maximum rate of absorption is soon followed by the maximum drop in blood sugar. Also, as the absorption rate drops to very low values, the blood sugar rises to its original level. There is still a small but measurable activity when the blood sugar has risen to its original level. This may be due to the presence of some denatured insulin.

Preliminary experiments were conducted on the distribution of the radioactive insulin, in rats injected intravenously and intracardially. An hour after injection the circulating blood contained a considerable fraction of the radioactive material. Relatively large quantities of radioactive material were found in the liver and kidneys, suggesting concentration of insulin by these organs.

Since part of the azo groups may be split off from the insulin by reduction in the body, distribution experiments of this type can be of value with regard to the physiology of insulin only if the rate of decomposition of the insulin derivative is also determined. However, as the reduction of azo compounds is relatively slow, it seems probable that in short experiments the distribution of the label would reflect the distribution of insulin in the body.

The technique described above represents a simple means for testing the rate of absorption of insulin depot preparations such as globin and protamine insulin.

L. REINER

ALBERT S. KESTON

M. GREEN

EXPERIMENTAL RESEARCH LABORATORIES,
BURROUGHS WELLCOME AND Co., U. S. A.,
TUCKAHOE, N. Y.

AND

DEPARTMENT OF BIOCHEMISTRY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY

COLCHICINE INDUCED UNIVALENTS IN DIPLOID ANTIRRHINUM MAJUS L.

THE spindle inhibiting effect of colchicine in mitosis and meiosis in both animals and plants is well known. However, the extent to which colchicine may affect the chromosomes themselves is less definitely established. Spiralization seems to be influenced by colchicine and a fusion or stickiness of the chromosomes frequently follows colchicine treatment. A low frequency of induced chromosomal aberrations and an altered mutation rate may also result. A disturbing effect on chromosome pairing and crossing over has also been reported. Walker,¹ Levan² and Dermen³ found uni-

¹ R. I. Walker, *Amer. Jour. Bot.*, 25: 280-285, 1938.

² A. Levan, *Hereditas*, 25: 9-26, 1939.

³ H. Dermen, *Jour. Hered.*, 29: 211-229, 1938.

valents a few days after treatment which they attribute to asynapsis or desynapsis. On the other hand, Darlington⁴ 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

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
	Percent- age ..	97.387	1.242	1.345	0	0.026	100
Treated	Actual.	6596	96	144	4	1	6,841
	Percent- age ..	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.

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.

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

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

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

ARNOLD H. SPARROW,
National Research Fellow

BIOLOGICAL LABORATORIES,
HARVARD UNIVERSITY

CRYSTALLIZATION OF A PROTEIN FROM POLIOMYELITIS INFECTED MOUSE BRAIN¹

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

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.