7. MATTHEWS, F. W., and MICHELL, J. H. *Ibid.*, **18**, 662 (1946).
8. WHITMORE, F. C. *Organic Chemistry.* New York: Van Nostrand, 29 (1937).
9. SHRINER, R. L., and FUSON, R. C. *Identification of Organic Compounds.* New York: Wiley, 101 (1940).
10. DUNSTON, A. E. *Science of Petroleum,* Vol. 2. Oxford: University Press, 1350 (1938).
11. DOSS, M. P. *Physical Constants of the Principal Hydrocarbons,* 4th ed. New York: Texas Co. (1943).
12. NOORDUYN, A. C. *Rec. trav. chim.,* **38**, 317 (1919).
13. MESSER, W. E. *Chem. News,* **138**, 292 (1929).
14. RASMUSSEN, R. S., BRATTAIN, R. R., and LUCCO, P. S. *J. Chem. Phys.,* **15**, 135 (1947).
15. FRED, M., and PUTSCHER, R. *Anal. Chemi.,* **21**, 900 (1949).

Manuscript received April 23, 1952.

Frequency of Dicentric Bridges in Meiosis in the Grasshopper *Gesonia punctifrons,* Produced by Different Dosages of X-Rays[1]

S. P. Ray-Chaudhuri and Ira Sarkar[2]

Cytogenetics Laboratory, Zoology Department, University of Calcutta, India

Experimental work on the effects of x-rays on the chromosomes of both animals and plants has been carried out mainly on the mitotic chromosomes. Similar studies on meiosis (*1–4*) have sometimes yielded contradictory results. In animals, especially, the studies on irradiated meiotic chromosomes (*5–11*) are not very numerous, and it seems desirable to have more quantitative data on the induced aberrations, obtained by direct cytological examinations conducted on the divisions immediately following treatment. We have, therefore, made a large-scale attempt to secure such data, as they seem to us to be essential not only for the understanding of the nature and causes of structural changes in the karyotype during evolution, but also for their special significance in relation to the effects of ionizing radiation. In the present paper we shall restrict ourselves to the presentation of the data of only one class of abnormality among several others found—namely, the frequency of dicentric bridges with fragments. These were detected at the first anaphase of meiosis by irradiating the testes of the grasshopper *Gesonia punctifrons,* a species with 23 acrocentric chromosomes in the males (Table 1).

Six experiments were carried out, with irradiations on the following dates: I, 5/12/48; II, 6/21/49; III, 4/3/50; IV, 5/9/50; V, 8/20/50; VI, 10/9/50. The grasshoppers to be treated with a particular dosage were put in separate muslin bags 4 × 5 cm in size. Two or three such bags, depending on the number of doses to be given in a particular experiment, were placed one upon another at a distance of 50 cm from the target. They were removed one by one at predetermined intervals. Radiation was carried out in the Radiology Department of the Calcutta Medical College, and the source of the x-rays was a G-E Maximar therapy tube. The voltage used was 200 kv, and the current was kept constant at 10 ma. The radiation was filtered through .5 mm copper and 1 mm aluminium filters. The dosages were varied by altering the time of exposure alone. It was not possible to measure the dosages in r-units in every experiment separately because the usual practice in this hospital is to calibrate the output of the tube at certain intervals of days. The various dosages indicated are therefore approximate, and errors of total dosages received in different experiments may be of the order of 4–5%.

The dosages used and the intervals between irradiation and treatment are shown for Expts I and II in Tables 2 and 3, respectively. In Expt III the only dosage was 80 r, the only interval 26 hr; in IV the doses were 40 r and 80 r, and the intervals 30 and 48 hr; in both V and VI the dosages were 40 r, 80 r, and 120 r, and the interval was always 30 hr.

G. punctifrons has been frequently used in our laboratory as experimental material and for the study of normal meiosis. Dicentric bridges were never observed in unirradiated material. It was therefore decided to have a single lot of grasshoppers to serve as controls, and the data for unirradiated material come from this lot alone.

TABLE 1

FREQUENCY OF DICENTRIC BRIDGES IN DIFFERENT EXPERIMENTS AT 30 HR AFTER IRRADIATION

Expt	40 r		80 r		120 r	
	Chromosomes	Bridges	Chromosomes	Bridges	Chromosomes	Bridges
I*	690	4			2,507	36
II	1,311	5	966	8		
IV	1,679	10	2,392	25		
V	1,794	7	2,093	16	4,278	47
VI	6,279	22	4,554	32	2,622	31
Sum	11,753	48	10,005	81	9,407	114
%		.41		.81		1.21
χ^2		2.50		2.36		1.53
P		.7		.5		.5

* Expt I, 280 r: 368 chromosomes, 17 bridges, 4.6%; Expt I, 320 r: 1,311 chromosomes, 47 bridges, 3.6%.

The frequencies of dicentric bridges accompanied by fragments in the first anaphase of meiosis, obtained in the different experiments when examined at 30 hr after irradiation, are shown in Table 1. It will be clear from the data that the frequencies found in the different experiments, with the same dosage, remain more or less constant, as the total χ^2 values indicate in each case. The corresponding value of P is not significant in any of the dosages, and therefore the slight deviations in the frequencies obtained during several repe-

[1] The authors record their deep sense of indebtedness to H. J. Muller for valuable suggestions incorporated in this paper. Thanks are also extended to S. Mukherji, professor of radiology, Calcutta Medical College, for providing facilities for x-raying in his hospital.

[2] The work was supported by a research fellowship offered to Ira Sarkar by the National Institute of Sciences of India.

TABLE 2

FIRST ANAPHASE BRIDGES AT VARIOUS HOURS AFTER IRRADIATION IN EXPT I

Hours after irradiation when fixed	40 r		120 r		280 r		320 r	
	Chromosomes examined	Bridges	Chromosomes examined	Bridges	Chromosomes examined	Bridges	Chromosomes examined	Bridges
6	1,219	1	—	—	—	—	—	—
12	1,173	4	1,380	15	1,380	56	—	—
24	1,219	3	460	3	1,196	42	575	20
30	690	4	2,507	36	368	17	1,311	47
48	1,104	6	1,495	17	1,127	8	851	33
72	552	2	2,162	24	253	15	1,610	38
χ^2 and P	$\chi^2 = 5.30$; $P = .5$		$\chi^2 = 2.70$; $P = .7$		$\chi^2 = 34.78$; $P < 10^{-5}$		$\chi^2 = 5.73$; $P = .2$	

titions of the experiment are negligible. The χ^2 values for 280 r and 320 r could not be calculated, for obvious reasons.

The data presented in Table 2 give the number of bridges obtained in the first experiment when the fixation was done at various hours after irradiation at the different dosages. The data for 6 and 12 hr at the relatively higher dosages could not be scored because the "sticky" bridges and other abnormalities characteristic of the physiological effect of irradiation persist up to 12 hr with 320 r and up to 6 hr with 120 r and 280 r. The χ^2 values for each dosage shown at the bottom of the table were calculated on the assumption that the frequencies do not vary at different hours for a particular dose. Their low values and the correspondingly high values of P at all dosages except 280 r indicate that the frequency remains essentially the same within the time limits of the experiment. The very high value of χ^2 (34.78) with 280 r is entirely due to an abnormally low frequency of bridges obtained at 48 hr. The recalculated value of χ^2, if we omit the data for 48 hr, is 3.48, and for 3 degrees of freedom the value of P is 0.50 and therefore shows no significant differences.

Expt II was carried out primarily to determine whether the frequency of bridges remains the same when the hours of fixation are extended even farther. The data obtained are shown in Table 3. The value of χ^2 with 11 degrees of freedom at 40 r and 80 r is very low and leads to the conclusion that the frequency of bridges does not vary significantly from the mean of all the different hours at a particular dosage.

We have shown that the frequency of bridges does not vary significantly from one experiment to another when the examination is made at 30 hr after treatment (Table 1). We are therefore justified in adding up the results of different experiments for the various dosages. The data presented in Table 1 indicate that the percentage of bridges is directly proportional to the dosage of radiation. This conclusion can be further strengthened if we add up the data for all hours at each dose. This can be done because we know that the frequency of bridges remains more or less constant at different hours after irradiation (Tables 2 and 3). The pooled results of all experiments at all hours, for each dose, are shown in Table 4. The percentages of bridges are, in general, clearly proportional to the dose. A slightly higher frequency is observed at 280 r in Tables 1 and 4. This deviation from direct proportionality is not significant, however, because doses of 280 r and 320 r do not differ much relatively, and the standard errors of the percentages obtained at each of these doses are comparatively high, owing to their being based on the examination of relatively few chromosomes.

Our results for anaphase bridges can be taken as

TABLE 3

FIRST ANAPHASE BRIDGES AT VARIOUS HOURS AFTER IRRADIATION IN EXPERIMENT II

40 r*			80 r†		
Hours	Chromosomes	Bridges	Hours	Chromosomes	Bridges
10	736	4	18	529	4
18	874	6	30	966	8
26	1,380	3	34	644	6
30	1,311	5	38	782	2
34	1,173	9	42	874	7
42	2,346	13	54	828	7
50	621	1	58	253	2
54	1,012	2	66	644	4
58	2,139	8	92	1,058	7
74	1,081	5	108	460	4
92	1,150	5	140	2,783	19
108	1,173	6	148	736	8

* $\chi^2 = 8.29$; $P = .70$.
† $\chi^2 = 4.74$; $P = .95$.

TABLE 4

DOSAGE-FREQUENCY RELATIONSHIP OF THE FIRST ANAPHASE BRIDGES
(Total for all hours)

Dose (in r)	Total chromosomes examined	Total bridges	Percentage
40	30,705	124	0.40
80	21,267	166	0.78
120	14,304	173	1.21
280	4,324	138	3.19
320	4,347	138	3.17
0 (Controls)	4,289	Nil	Nil

agreeing closely enough with a relationship of direct linear proportionality of number of effects to dosage used. If these aberrations result from inversions or other changes involving two independent breaks, then we should as a first approximation expect the number of cases to vary as the square of the dose. The relationship would be somewhere between the first power and the square, however, if the radiation had to be extended over a time very much longer than it usually takes for a broken end derived from one break to find and unite with a broken end derived from another break. The time of treatment at different x-ray dosages in our experiment varied only between 1 and 14 min, and therefore, if the changes were due to two independent breaks, the frequencies found should in fact vary nearly as the square of the dose. In view of the statistics of our results we have to conclude that most of the bridges are not formed by independent breaks, but by one or more breaks caused by a single ionization or activation, or occurring in the course of a single ionizing track.

The hypothesis which appears to us most probable is that the breaks of the two chromatids which, on uniting with one another, form the dicentric chromosome of the first anaphase bridge, are both derived from one break, which occurred in a chromosome before its division into chromatids. This is the hypothesis which was proposed by Muller (*12*) to explain losses of single chromosomes that had been irradiated in the spermatozoon stage. It also corresponds in principle with the spontaneous breakage-fusion-bridge cycle found by McClintock (*13*) in maize. The sequence of events, as we conceive it to occur in our material, is represented in Fig. 1, *a* to *e*. It will be seen that this series of steps is similar in its later stages to that proposed by A. R. Whiting (*9, 10*) for the chromosome bridges found by her in the second meiotic anaphases of *Habrobracon* oocytes irradiated during the latter part of the first meiotic metaphase. Since, however, the chromatids were already in existence at the time of irradiation, it was clear that in her material each second anaphase bridge represented the effect of two breaks, one in each of two neighboring chromatids. It was inferred from the linear relation to dose that both breaks were caused by the same "hit."

The constancy of the frequency of bridges recorded at different hours after irradiation, up to a period of 148 hr, is a curious phenomenon. In grasshoppers, a long resting period intervenes between the last spermatogonial division and the onset of meiosis. The large majority of bridges which we detect between 12 and 148 hr at the lower dosages (the results at 6 hr being too few to be significant), and between 10 and 72 hr at relatively higher ones, probably originate in chromosome breaks induced by radiation during this resting period.

In the case of a small fraction of bridges in our material, especially those observed at less than 12 hr after treatment at higher doses, it may be that, as Whiting believes to happen in her material, they originate during the early prophase of meiosis, in pachytene or as late as early diplotene, by a single "hit" breaking both the sister chromatids; these cases, like the others, would have to undergo sister reunion later, so as to give rise to a bridge and fragment at anaphase I. According to Darlington and Koller (*14*), the regions between the chromomeres in the prophase of meiosis are relatively undercharged with nucleic acid; they believe that chromosome threads in this condition have breakages more readily induced in them, in these regions, by radiation.

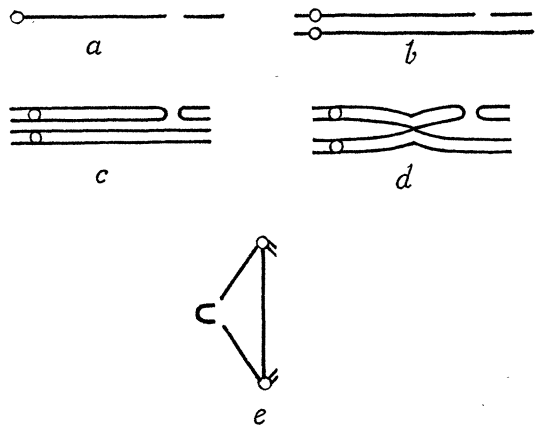

FIG. 1. *a*, A break in an unsplit chromosome; *b*, the broken chromosome pairing with its unbroken homologue; *c*, pachytene, after the reproduction of chromosomes, the centric and acentric chromatids having undergone sister reunion; *d*, a chiasma being formed between the centromere and the break; *e*, first anaphase producing a dicentric bridge and a fragment.

We do not think it likely, however, that the chromosomes have already divided into chromatids in the cells which were treated in the less mature stages dealt with in our material. If they have not, then for most of the breaks—those occurring according to the scheme presented in Fig. 1—bridge formation could take place only after the reproduction of the chromosomes at pachytene, by sister union of daughter chromatids derived from broken chromosomes. In that case, in order to explain, under our scheme of bridge formation, the constancy of its frequency for treatments given at widely different times before the first meiotic division, we must infer further that the breaks produced by x-rays in the resting spermatocytes are preserved for a long period—in extreme cases for nearly 148 hr—without undergoing restitution.

This long delay in union of broken ends was first found to hold for the chromosomes in the mature spermatozoa of *Drosophila,* in experiments participated in by Muller and the present senior author in collaboration (*12*). On the other hand, work of numerous authors has been regarded as showing that in most resting (interphase) stages which precede mitotic divisions, the union of broken ends can take place immediately after breakage, most of it being completed, according to Sax (*15*), within less than an hour thereafter. If this is true the conditions for joining of the broken ends during the premeiotic interphase in our material would be different from that generally ob-

served for mitotic interphases. Lane (*16*), however, in work which came to our attention after our interpretation of our own results had been arrived at, has recently concluded, on the basis of evidence obtained by him, that in *Tradescantia* microspores the union of broken ends is, contrary to the previously accepted interpretation for such material, long delayed. His conclusion is at present under dispute (*17, 18*).

References

1. Goodspeed, T. M. *J. Heredity,* **20**, 243 (1929).
2. Riley, H. P. *Cytologia,* **7**, 131 (1936).
3. Sax, K. *Genetics,* **23**, 494 (1938).
4. Stone, L. H. A. *Ann. Botany,* **47**, 815 (1933).
5. Bishop, D. W. *Genetics,* **27**, 132 (1942).
6. ———. *J. Morphol.,* **71**, 391 (1942).
7. Bozeman, M. L. *Genetics,* **28**, 71 (1943).
8. White, M. J. D. *Proc. Roy. Soc. (London)*, B **124**, 183 (1938).
9. Whiting, A. R. *Am. Naturalist,* **79**, 193 (1945).
10. ———. *Biol. Bull.,* **89**, 61 (1945).
11. Marshak, A. *J. Gen. Physiol.,* **19**, 179 (1935).
12. Muller, H. J. *J. Genetics,* **50**, 1 (1940).
13. McClintock, B. *Research Bull. Mo. Agr. Expt. Sta.,* No. 290 (1938).
14. Darlington, C. D., and Koller, P. C. *Heredity,* **1**, 187 (1947).
15. Sax, K. *Genetics,* **25**, 41 (1940).
16. Lane, G. R. *Heredity,* **5**, 1 (1951).
17. *Ibid.,* **6**, 131 (1952).
18. Sax, K. *Ibid.,* 127.

Manuscript received May 5, 1952.

Effect of a Cationic Detergent on the Digestion of Raw Cornstarch *in Vitro*[1]

Robert L. Gates and R. M. Sandstedt

Department of Agricultural Chemistry, Nebraska Experiment Station, University of Nebraska, Lincoln

Recent reports have indicated that certain detergents increase the growth rate of chickens (*1*), rats (*2*), and pigs (*3*). The mechanism of such growth stimulation is not understood, but it has been suggested that it is related to surface-active properties (*4*).

During a study of the digestion of raw cereal starches by α-amylases from various sources, it was noted that the presence of "Roccal,"[2] a mixture of high molecular weight alkyl-dimethyl-benzyl-ammonium chlorides, was effective in increasing the rate of action of the α-amylases from fungal, bacterial, and animal sources. This detergent was effective in increasing the activity of the amylases on the raw starches but not on cooked starches.

The exceedingly slow rate of digestion of raw starches is well known. It is possible that some of the energy value of a feed is not made available to the animal because of incomplete digestion during the relatively short period in the intestinal tract—an increase in the rate or extent of digestion should increase the efficiency of gain.

The data presented in Table 1 indicate the effectiveness of Roccal in increasing the rate of action of pancreatic amylase on raw starch. These data show that the increased rate of action was evident over a pH range from 5.4 to 7.8, but was most effective near the pH of maximum activity. Table 2 compares the effect of the detergent, an organic electrolyte, with two inorganic electrolytes that are known to stimulate the action of pancreatic amylase on cooked starch.

TABLE 1

Effect of 0.1% Roccal on the Digestion of 500 mg Raw Cornstarch by 4 mg Pancreatin*

pH	Raw starch digested		
	Without detergent (%)	With detergent (%)	Increase of activity (%)
5.4	25.7	27.6	7
6.2	37.2	54.1	45
7.0	37.1	58.3	57
7.8	31.8	51.2	57

* Twenty-hr digestions carried out at 30° C on 20 ml 2.5% raw cornstarch suspension, buffered with phosphate and constantly agitated.

TABLE 2

Electrolyte Stimulation of the Digestion of 500 mg Raw Cornstarch by 1.5 mg Pancreatin, pH 6.5*

Digestion condition	Raw starch digested	
	Amount (%)	Increase of activity (%)
Without detergent		
Control	28	—
0.05% NaCl	37	32
0.10% NaCl	38	35
0.10% $CaCl_2$	37	32
With 0.1% detergent		
Control	43	54
0.05% NaCl	45	61
0.10% NaCl	46	64
0.10% $CaCl_2$	56	100

* Twenty-hr digestions carried out at 30° C on 20 ml 2.5% raw cornstarch suspension, buffered with phosphate and constantly agitated.

The demonstrated stimulation of pancreatic α-amylase by calcium or sodium chlorides, and the stimulation of raw starch digestion by Roccal, suggest that these two effects may be the same or similar in mechanism. To obtain information concerning the action of these two types of enzyme stimulant a series of determinations was made in which the stimulants were studied individually and in combination on raw starch digestion. Digestions were carried out with 0.10% Roccal alone, with .05% and .10% sodium chloride, and 0.10% calcium chloride, both individually and in combination with the 0.10% Roccal, each digest being

[1] Preliminary report; published with the approval of the director as Paper No. 562, Journal Series, Nebraska Agricultural Experiment Station.

[2] Roccal is the brand name of a product sold by Sterwin Chemicals, Inc., New York 18.