

also base pair substitutions in *Neurospora*. In tests on *Vicia faba* chromosomes, ICR-170 has been shown to be a potent chromosome-breaking agent inducing chromatid and isochromatid deletions, and subchromatid, chromatid and chromosome exchanges⁷. The experiments reported here show that inversions result in the salivary gland chromosomes of *Drosophila* after treatment with ICR-170.

Freshly laid eggs of Oregon-K strain were plated on the ICR-170 medium. This medium was prepared by mixing equal volumes of 200 μ g of ICR-170 per ml of distilled water and basic medium. Basic medium was composed of agar (3%), yeast (10%), glucose (10%), propionic acid (0.4%) and water (100 ml). For the control, eggs were plated on the basic medium diluted with distilled water (1:1). Third instar larvae from control and experimental media were harvested for studying salivary gland chromosomes. The treatments were conducted in darkness to eliminate the possibility of the photodynamic action of the acridine nucleus. Salivary glands were excised in saline water and squashed immediately in aceto orcein. (ICR-170 was the kind gift from Dr. H. J. CREECH, of the Institute for Cancer Research, Philadelphia, USA.)

4 spontaneous and 52 ICR-170-induced inversions were recovered in 500 cells scored for control and treated larvae. The inversion frequency per cell works out at 0.008 and 0.104 for control and treated larvae respectively. Most of the ICR-170-induced inversions were complex. The concentration of ICR-170 used in these experiments yielded 5.8% sex linked lethals.

ICR-170 has 2 components: an acridine nucleus and a monofunctional nitrogen mustard side chain. Mutagenicity of acridines in bacteriophages is related to acridine intercalation⁸ and that of alkylating agents to reaction of agents with nitrogenous bases of DNA, primarily by alkylation of guanine at the N7 position⁹. Whereas the alkylating agents are potent chromosome-breaking

agents^{7,10-12}, the acridines have a milder action and that only under photodynamic conditions¹³. From the specificity of reversions of frameshift, amber, ochre and missense types of mutations in the *his C* gene of *Salmonella*, it is suspected that ICR-170, at low concentrations, may be acting only by the acridine nucleus and not by the alkylating side chain⁵. Chromosome aberration induction studies have led us to suspect that alkylating chain is necessary for its induction of lesions in chromosomes⁷.

Zusammenfassung. Bei Kontrolle der Speicheldrüsen ergibt sich, dass die Acridinbehandlung bei *Drosophila* Komplexinversionen erzeugt.

SUSHIL KUMAR and R. P. SHARMA

Division of Genetics, Indian Agricultural Research Institute, New Delhi 12, (India), 23rd August 1966.

¹ E. A. CARLSON and I. I. OSTER, *Genetics* 47, 561 (1962).

² J. L. SOUTHIN, *Mutation Res.* 3, 54 (1966).

³ L. A. SNYDER and I. I. OSTER, *Mutation Res.* 1, 437 (1964).

⁴ H. E. BROCKMAN and W. GOBEN, *Science* 147, 750 (1965).

⁵ B. AMES and H. WHITFIELD JR., *Cold Spring Harb. Symp. quant. Biol.*, in press (1966).

⁶ H. J. CREECH, E. BREUNINGER, R. F. HANKWITZ, G. POLSKY and M. I. WILSON, *Cancer Research* 20, 471 (1960).

⁷ S. KUMAR, U. AGGARWAL and M. S. SWAMINATHAN, *Mutation Res.*, in press (1966).

⁸ S. BRENNER, L. BARNETT, F. H. C. CRICK and A. ORGEL, *J. molec. Biol.* 3, 121 (1961).

⁹ P. BROOKS and P. D. LAWLEY, *Biochem. J.* 80, 496 (1961).

¹⁰ B. A. KIHLMAN, *Expl Cell Res.* 20, 657 (1960).

¹¹ B. A. KIHLMAN, *Mutation Res.* 7, 54 (1964).

¹² M. M. COHEN and M. W. SHAW, *J. Cell Biol.* 23, 386 (1964).

¹³ S. KUMAR and A. T. NATARAJAN, *Mutation Res.* 2, 11 (1965).

Elective Localization of ³H-Corticosterone in Mast Cells

Following glucocorticoid treatment, the number of mast cells increases¹⁻³; this result, however, is not merely quantitative but also qualitative, leading to the accumulation of the granules of the mast cells, which finally disintegrate⁴. At this phase the observable number of mast cells recedes. This physiological control of the formation and disintegration of mast cells effected by glucocorticoids seems fairly obvious; nothing is known, however, of the cellular mechanism of this control.

Mast cell transformation can be followed easily in subcutaneous connective tissue⁴ but still better in the lymphatic organs^{1,2,5-7}. Here some cells – reticular cells and lymphoblasts – transform into mast cells. It is not known whether this is in any way connected with the cortisone-cortisol transformation observable in the lymphatic organs. As is known from the experiments of DOUGHERTY et al.⁸, the system of 11 β -hydroxydehydrogenase is present in the lymphatic organs and, during a prolonged cortisone treatment, activity of the enzyme significantly increases in the thymus. This can be hypothetically connected with the simultaneous appearance of this massive mast cell formation.

Since the adrenal cortex of rats and mice produces first and foremost corticosterone⁹, the adult male BALB/C mice used in our experiments were given corticosterone-1,2-³H. The specific activity of corticosterone was 945.9 mCi/mM. Labelled corticosterone was diluted with a non-labelled one, and after its suspension with Tween 80 was injected i.p. Thus 1 mg/30 g of corticosterone was given, one tenth of which was active. The activity given was 5 μ Ci/g body weight. 6 animals were treated in this manner. Material was taken from 2–2 animals 1, 3 and 6 h following treatment. The apex of

¹ L. ARVY, J. A. BOIFFARD and M. GABE, *C. r. Soc. Biol.* 146, 1537 (1952).

² L. ARVY, J. A. BOIFFARD and M. GABE, *Revue Hémat.* 9, 454 (1954).

³ H. SELYE, *The Mast Cells* (Butterworths, Washington 1965).

⁴ G. CSABA and L. KULLMANN, *Acta biol. hung.* 14, 175 (1963).

⁵ G. CSABA and K. MOLD, *Z. mikrosk.-anat. Forsch.* 68, 163 (1962).

⁶ G. CSABA, I. TÖRÖ and M. BODOKY, *Z. mikrosk.-anat. Forsch.* 69, 467 (1963).

⁷ G. CSABA, I. TÖRÖ and M. BODOKY, *Acta Anat.* 67, 127 (1965).

⁸ T. F. DOUGHERTY, M. L. BERLINER and D. L. BERLINER, *Endocrinology* 66, 550 (1960).

the heart of the etherized animal was cut off and a cannula was inserted into the aorta. Dextran solution (Plasmadex, Human), then buffered (phosphate buffer pH 7.2) 4.5% glutaraldehyde (Fluka) was perfused through the cannula. (The perfused glutaraldehyde fixation ensures the possibility of a subsequent electron microscopic autoradiography.) The thymus, spleen and axillary lymph nodes of the animals were removed, embedded, and 10 μ sections were made. A part of the sections was covered by Kodak AR 10 stripping film and after an 8 week exposure at 4°C were developed in Kodak D 19 developer at 18–20°C for 5 min. The other sections were stained with PAS and toluidin blue. These served

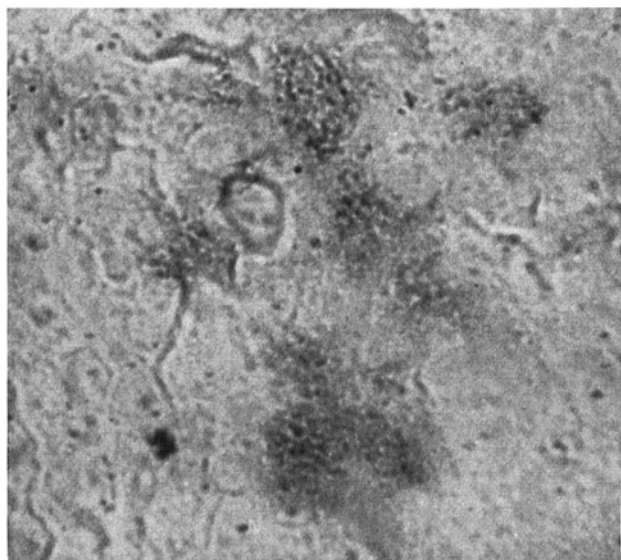


Fig. 1. Labelled cells in the cortex of the thymus appearing PAS positive in the parallel section, 3 h following ^3H corticosterone treatment. Unstained, oil immersion, $\times 450$.

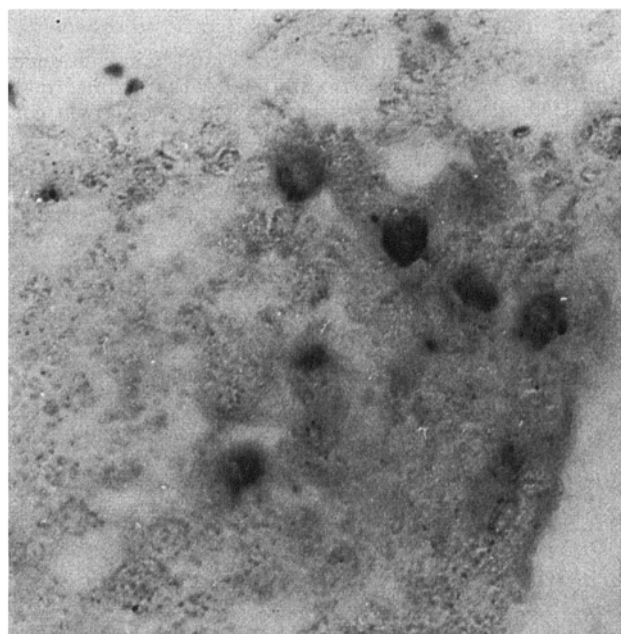


Fig. 2. Labelled mast cell group in lymph node, 6 h after treatment. Unstained, $\times 180$.

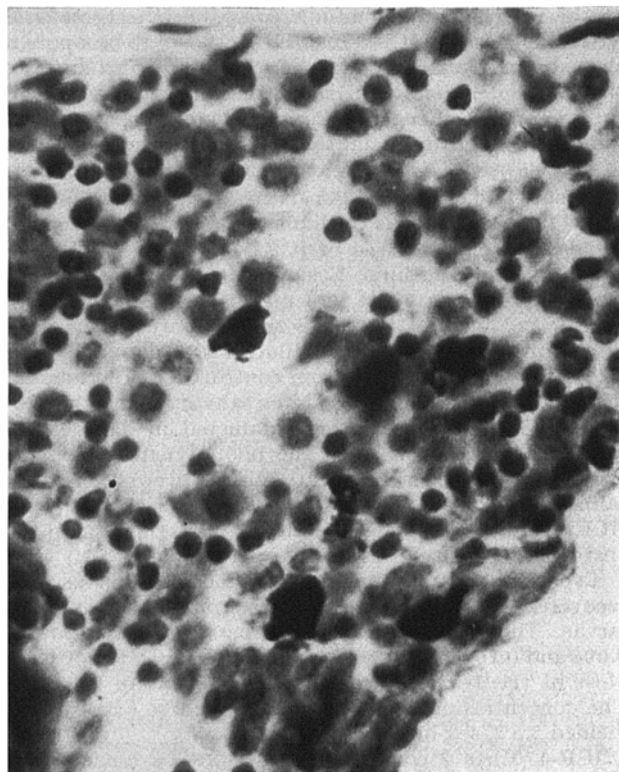


Fig. 3. The same mast cell group in the parallel preparation. Toluidin blue, $\times 240$.

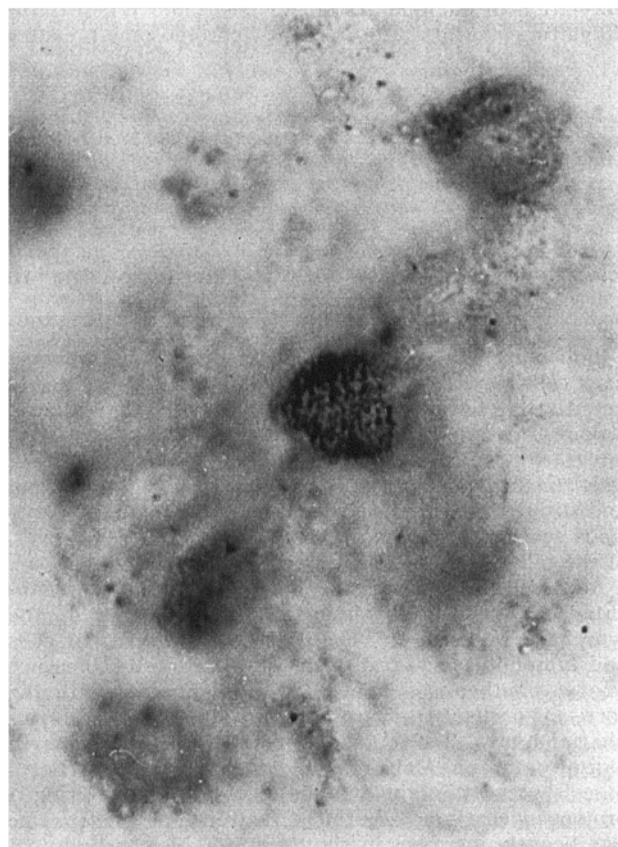


Fig. 4. 4 cells from the mast cell group of Figure 2. Unstained, oil immersion, $\times 450$.

as parallel sections. Some of the autoradiographic preparations were stained with PAS + azur; the others, after mounting, were studied unstained.

1 h following treatment, slight activity is present in some of the PAS positive cells of the thymus. After 3 h, PAS positive cells localized around the cortical vessels of the thymus showed high activity (Figure 1). A few PAS positive cells are present also in the lymph nodes, while the spleen is absolutely negative. After 6 h, very high activity can be seen in the mast cells of the lymph nodes. The mast cells are localized mainly in the sinuses in groups. Activity is localized exclusively to the mast cells and not perceivable in other cells. Although activity is well observable on the stained preparations, since the mast cell itself appears granular in stained preparations, a comparison of the unstained preparations with the parallel ones appears to be more promising (Figures 2, 3 and 4). It is easily observable that localized activity in the identifiable cells conforms with the stained mast cell groups.

Thus the experiment shows that the PAS positive cells – which in other experiments were observed as developing forms of the mast cells – and mast cells, electively take up ^3H corticosterone. No explanation can be given for the why and wherefore of this phenomenon. It is possible that, as heparin inhibits the reduction

of corticosterone⁹, the hormone is retained only in the mast cells. But this does not seem probable as activity in the mast cells increases just after 6 h. On the other hand, if corticosterone does disintegrate, isotope activity could be still present, which suggests an active uptake, or rather an active uptake taking place during transformation into mast cells. Should this indeed be the case, this would present the glucocorticoid-mast cell relation from an entirely different aspect.

Zusammenfassung. Die Aufnahme von Corticosteron-1,2- ^3H in lymphatische Organe von Mäusen des Stammes BALB/C wurde untersucht. Autoradiographisch wurde gefunden, dass das markierte Corticosteron elektiv in den PAS-positiven Zellen bzw. später in den Mastzellen erscheint.

G. CSABA, J. KISS and C. DUNAY

*Institute of Histology and Embryology,
Medical University, Budapest (Hungary),
28th October 1966.*

⁹ R. C. TROOP and J. T. BIGGS JR., *Metabolism* 14, 867 (1965).

Nucleic Acid Content of a Diploid and of a Triploid Strain of *Drosophila melanogaster*

The size of diploid individuals of *Drosophila melanogaster* is similar to that of triploid ones. These animals obviously possess a regulating system that adjusts the action of the 3 genomes of the triploids to almost the same action of the 2 genomes of the diploids. Since cytological experiments with giant chromosomes suggest that this adjustment takes place on the chromosomal level, the nucleic acid content of diploid and triploid *Drosophila* strains have been determined quantitatively. The results are presented in this paper.

The objects used in this experiment were larvae of the last stage of the diploid strain 'Berlin normal' and of the triploid strain of the genetic constitution $FM4, y^{31d} sc^8 dm B/y^2 sc w^a ec.=^1$. The animals were cultured under standard conditions². In the triploid strain, at best half of the larvae are triploid, so that besides triploids always at least as many diploid larvae have been used for determination of nucleic acid content. The total amount of nucleic acids, extracted according to SCHNEIDER³, has been measured by UV-absorption⁴. The DNA and RNA contents have been determined according to BURTON⁵ and CERIOTTI⁶ respectively.

The investigation has been carried out on 2 separate lines (29 single experiments); the results of both are almost in agreement. In this paper only the data of 1 of the 2 lines will be presented (Table).

The weight of 100 larvae of the triploid strain is about 10% lower than that of the diploids, and the portion of the lipid-free dry weight in respect of the wet weight is about 4% lower in the triploid strain than in the diploid one. It is striking that these differences are in contrast to the number of genomes.

The total nucleic acid content as well as the RNA content shows no significant differences in either strain. The DNA content of the triploid strain, however, is about 15% higher than that of the diploid one. Therefore the DNA/RNA ratio is changed too. The diploid strain shows a ratio of about 1:25, the triploid strain a ratio of 1:20. More detailed investigations of the diploid strain have shown that the ratio is constant even if the size and weight of the larvae are modified by environmental factors.

Provided that the portion of the triploid larvae of the triploid strain is 50%, one would expect about 25% more DNA in the triploid strain than in the diploid one. But, from the amount expected, only $\frac{2}{3}$ are measured. Since in the experiments the % of $3n$ - and $2n$ -larvae could not be examined, one has to suppose that the deficit of DNA is correlated to a lower amount of triploid larvae. But neither can one exclude the possibility that the amount of DNA of 3 genomes in the triploid individuals is less than one would expect.

Since, in spite of the high DNA content, the RNA content in the triploid strain is as high as in the diploid one, the 3 genomes of the triploids are as active as the 2 genomes of the diploid larvae – assuming equal stability of RNA in both strains.

The regulating mechanism which makes the size of these triploid organisms similar to that of the diploid

¹ We wish to thank Dr. I. OSTER (Bowling Green, Ohio) for kindly providing the triploid strain.

² F. MAINX, *Das kleine Drosophila-Praktikum* (Springer, Wien 1949).

³ W. C. SCHNEIDER, *J. biol. Chem.* 161, 293 (1945).

⁴ M. OGUR and G. ROSEN, *Archs Biochem.* 25, 262 (1950).

⁵ K. BURTON, *Biochem. J.* 62, 315 (1956).

⁶ G. CERIOTTI, *J. biol. Chem.* 214, 59 (1955).