possède que 22 chromosomes. Le couple hétérochromosomique, très curieux et analogue à celui de *Mesocricetus*, sera analysé dans un mémoire ultérieur consacré à la question de la préréduction et de la postréduction. Disons ici que l'Y a la forme d'un petit V, morphologie qui se retrouve dans la région proximale de l'X. Mais l'un des bras de celui-ci se prolonge, à la métaphase I, en un long filament hétérochromatique déspiralisé. Le nombre diploïde 22 est très bas pour un euthérien: 8, peut-être 9 paires d'éléments sont méta- ou submétacentriques; les deux ou trois autres trop petites pour que le type d'attachement puisse être élucidé avec certitude. Le «nombre fondamental» est donc voisin de 40, égal au minimum à 38. Or, chez *Cricetulus griseus* M. E., PONTECORVO[1] a compté 14 chromosomes seulement. J'ai discuté ce cas[2] et mis en évidence la mauvaise qualité du matériel utilisé, ce qui, à mon sens, rendait très douteuse la réalité des observations de PONTECORVO. Cependant, le petit nombre diploïde de *Cricetus cricetus* reposait le problème et je me suis demandé si ma critique était bien fondée. Grâce à la générosité de M. V. SCHWENTKER qui s'est voué à Tumblebrook-Farm (N. Y.) à l'élevage de petits Mammifères destinés aux recherches scientifiques, j'ai pu recevoir et étudier le *Cricetulus griseus* chinois. La formule de cet intéressant rongeur est très semblable à celle du hamster européen et le nombre diploïde également de 22. Ainsi disparaît de la littérature une donnée très insolite et dont, dès 1949, j'avais souligné l'invraisemblance. Nous ne connaissons pas, à l'heure actuelle, d'euthérien ayant moins de 22 chromosomes. R. MATTHEY

Laboratoire de zoologie de l'Université de Lausanne, le 29 juin 1951.

Summary

Microtus pennsylvanicus does not represent a twin-species of *Microtus agrestis*. Its formula is $2N = 46$. X and Y are of usual size.—*Microtus guentheri* has the following chromosome-number: $2N = 54$. All the autosomes are acrocentric. X and Y are small and both metacentric.—*Mesocricetus auratus* shows effectively 44 (HUSTED and co-workers) and not 38 (KOLLER, MULDAL) chromosomes.—*Cricetus cricetus* has a very low number ($2N = 22$) of chromosomes and peculiar sex-chromosomes like those of *Mesocricetus*.—*Cricetulus griseus* has the same diploid number, $2N = 22$. The observations of PONTECORVO (1943) on this species may now be discarded.

[1] G. PONTECORVO, Proc. Royal. Soc. Edinburgh [B] *62*, 32 (1943).
[2] R. MATTHEY, *Les chromosomes des Vertébrés* (Edition Rouge, Lausanne 1949).

The Effect of Oxygen Concentration on the Mutagenic Action of Mustard Gas

It is now a well-established fact that lowering of the oxygen tension during X-radiation reduces the frequency of genetical changes in the exposed cells[1]. In particular, it has been shown[2] that there is a striking reduction in the frequency of X-ray induced sex-linked lethals in *Drosophila melanogaster* when irradiation is carried out in an atmosphere of nitrogen. The interpretation of this phenomenon is still doubtful; but it is generally assumed that the shortage of oxygen in irradiated cells interferes with one of the processes through which ionizing energy is transferred to the chromosomes, such as the formation of H_2O_2, free water radicals, or peroxides.

It seems, however, conceivable that in these experiments oxygen acts not so much through influencing the specific course of radiation induced mutation, as through some more general, physiological process which affects sensitivity of the chromosomes to whatever mutagenic influence happens to impinge on them. In order to explore this possibility, two experiments were carried out in which *Drosophila* ♂♂ were exposed to mustard gas in an oxygen or nitrogen atmosphere. In both experiments, the flies for the two kinds of exposure came from the same culture bottles and were of the same age. Exposure was carried out in an apparatus designed by one of us (H. M.). Its essential feature is the exposure of the flies at constant temperature to a regulated flow of air which has passed over liquid mustard gas. In the present experiment, the air in the mustard gas flask was first replaced by either oxygen or nitrogen, and the flies were exposed for ten minutes to a preliminary flow of the pure gas. Subsequently, the mustard gas flask was connected with the flies' vial, and the mixture of mustard gas with either oxygen or nitrogen was sucked through this vial for three minutes; the taps leading to the flies' vial were then closed, and exposure was continued in the closed vial without further flow of gas for another six minutes. Treatment was followed by ten minutes' exposure to a flow of pure oxygen or nitrogen. Temperature and rate of flow were, of course, identical in the two series which made up each experiment. Both exposures were given on the same day, separated from each other only by the time interval required for decontamination, re-filling of the mustard gas flask, and pre-treatment of the flies with the pure gas to be used in the second series. In the first experiment it was noticed that the flies exposed in nitrogen became anaesthetized more speedily than those exposed in oxygen. In order to avoid differences in the effectiveness of the treatment which may result from different degrees of movement during exposure[1], the flies for the second experiment were etherized before use. They remained anaesthetized during the whole period of pre-treatment, exposure, post-treatment, and decontamination. The second experiment was more accurate than the first in still another respect: ♂♂ from this experiment were mated to a succession of fresh virgin ♀♀ on the same days in both series, and the F_1 ♀♀ for the subsequent genetical tests were chosen so as to contain comparable proportions of each brood in both series; since it has been shown that mustard gas acts differentially on the various stages of sperm development[2], this precaution is necessary for accurate quantitative comparisons of mutation rates.

The table shows that mutation rates in both experiments were unaffected by the type of gas used.

In the first experiment, the difference between the mutation rates in nitrogen and oxygen is not significant. In the second, more accurate one, the two mutation rates are practically identical.

[1] J. M. THODAY and J. READ, Nature *160*, 608 (1947); *163*, 133 (1949). – L. SMITH, R. S. CALDECOTT, and B. HAYDEN, Genetics *33*, 629 (1948); *34*, 26 (1949). – N. H. GILES, Jr. and H. P. RILEY, Proc. Nat. Ac. Sc. U.S.A. *35*, 640 (1949); *36*, 337 (1950); Genetics *35*, 131 (1950). – N. H. GILES and A. V. BEATTY, Genetics *35*, 666 (1950); Science *112*, 643 (1950). – A. C. FABERGÉ, Genetics *35*, 663 (1950). –
[2] W. K. BAKER and E. SGOURAKIS, Proc. Nat. Ac. Sc. U.S.A. *36*, 176 (1950).

[1] C. AUERBACH and J. M. ROBSON, Proc. Roy. Soc. Edin. *62*, B 271 (1947).
[2] C. AUERBACH, Proc. Eighth Int. Congr. Genetics *128* (1949); Pubbl. Staz. Zool. Napoli *22*, Suppl. 1 (1950) – B. P. KAUFMANN, H. GAY, and H. ROTHBERG jr., J. Exp. Zool. *111*, 415 (1949).

Frequency of sex-linked lethals in the progeny of ♂♂ which had been exposed to the same dose of mustard gas in (*a*) nitrogen, (*b*) oxygen.

Exp.	*Nitrogen*			*Oxygen*		
	No. chromosomes tested	Lethals		No. chromosomes tested	Lethals	
		No.	%		No.	%
I	415	26	6·2	602	32	5·3
II	687	68	9·9	520	54	10·4

In spite of all the precautions used it is not possible to affirm that the amount of mustard gas administered in the two series of one experiment was identical. However, the difference can have been only slight, and it seems safe to conclude that, should oxygen exercise an effect on the production of mutations by mustard gas, this effect cannot be of the same order of size as observed for X-radiation. This lends support to the view which considers that the contrasting results obtained with irradiation indicate that oxygen is involved specifically in the production of mutations by X-rays.

We want to acknowledge gratefully the help of the Honours and Diploma class which in the spring term 1951 carried out the genetical part of the first experiment.

C. AUERBACH and H. MOSER

Institute of Animal Genetics, University of Edinburgh, May 31, 1951.

Zusammenfassung

Senfgasbehandlung von *Drosophila*-Männchen wurde in einer Atmosphäre von reinem Sauerstoff und von reinem Stickstoff vorgenommen. Die mutagene Wirkung erwies sich quantitativ als unabhängig von dem benutzten reinen Gas. Dieses Resultat stellt einen Gegensatz dar zu ähnlichen Versuchen mit Röntgenstrahlen und deutet auf einen Unterschied in der primären mutagenen Wirkung dieser beiden Agentien hin.

The Use of Solvents in Tissue Cultures

In a series of investigations we have tried to evaluate in tissue cultures the antimitotic activity of some quinones and related compounds[1]. So far, we have used for this purpose only water-soluble substances. No adequate method for water-insoluble substances was available. Previous investigators have tried to overcome this difficulty by working with acetone or alcohol as solvents. Both solvents may influence the cells in mitosis and, or resting cells, as pointed out by v. MÖLLENDORFF[2] and by BUCHER[3], and in spite of a number of controls the results remain unsatisfactory in tissue cultures.

As we had to deal repeatedly with water-insoluble substances our experiments remained incomplete. We had to look, therefore, for ways of filling the gaps of our investigations. A suitable solvent was found in ethylene glycol monoethylether (Cellosolve). We combined it with gum ghatti, introduced by FOLIN[4], for biochemical work and used more recently by ANSON[5] and by MIRSKY[6].

We dissolved the substance to be tested in Cellosolve, added the same volume of a preparation containing 2% of sterile gum ghatti (B.D.H.) to give a 2×10^{-3} M solution of our compound. The mixture was diluted with sterile Tyrode fluid to a molar concentration of 2×10^{-5} which was mixed 1:1 with embryo extract. This was the highest concentration used in tissue cultures with these compounds. It contained the substance at 1×10^{-5} M concentration, further 0·25% Cellosolve and 0·25% of a 2% solution of gum ghatti.

Controls carried out in tissue cultures of chick fibroblasts with a mixture containing 0·25% Cellosolve and 0·25% of a 2% solution of gum ghatti gave no inhibition of mitosis, and no abnormal dividing cells or resting cells were observed. In contrast the corresponding mixtures prepared with acetone instead of Cellosolve showed a great number of clumped metaphases, some fragmentations and undivided telophases.

A number of water-insoluble substances have been tested in tissue cultures with the described method. Satisfactory results were obtained. These experiments will be reported elsewhere.

One of us (E. F.) is indebted to Messrs. MAY & BAKER Ltd., Dagenham, for financial support.

E. FRIEDMANN and (Mrs.) I. SIMON-REUSS

Department of Radiotherapeutics, University of Cambridge, England, May 9, 1951.

Zusammenfassung

Für die Prüfung wasserunlöslicher Substanzen in Gewebskulturen hat sich eine Lösung der Substanzen in Äthylen-glykol-monoäthylester (Cellosolve) und Zusatz des gleichen Volumens steriler 2%iger Gummi-ghatti-Lösung bewährt. Die Arbeitsweise zur Herstellung entsprechend verdünnter Lösungen, wie sie in Gewebskulturen benutzt werden, wird beschrieben.

Purines libres et mononucléotides dans l'embryon de batracien

De récentes observations[1] semblent confirmer une hypothèse émise par BRACHET[2] selon laquelle les phénomènes d'induction, chez les embryons de batraciens, seraient accompagnés d'une migration d'acide ribonucléique de l'inducteur vers le neuroblaste; cet acide nucléique se déplacerait en liaison avec des structures cytoplasmiques et non sous la forme de mononucléotides. Il nous a paru utile cependant de rechercher si, au stade du développement où se fait l'induction, il ne serait pas possible de déceler une libération de mononucléotides.

Après quelques essais, nous avons adopté une technique fort simple de dosage, basée sur l'absorption dans l'ultra-violet des purines ou composés puriques acido-solubles et sur l'analyse de ces substances par chromatographie sur papier. Les embryons sont homogénéisés et extraits deux fois à l'acide perchlorique 1% à 0°C. Cet extrait brut, même après centrifugation, présente toujours un léger trouble qui ne disparaît qu'après une agitation vigoureuse avec un égal volume de chloroforme. Nous avons pu vérifier qu'au cours de cette opération la totalité des purines reste dans la phase aqueuse. Le spectre d'absorption de ces extraits est déterminé, entre

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[2] W. v. MÖLLENDORF, Klin. *18*, 1098 (1939).

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