Par contre si, comme les souris normales, les souris obèses hypothalamiques réagissent au jeûne par une augmentation de la cétonémie, les souris héréditairement obèses réagissent au jeûne par une diminution de la cétonémie, une réaction qui peut s'ajouter à celles qui différencient les obésités «de métabolisme» des obésités «de régulation».

Nuclear Chimaeras in the Newt

Direct tests as to whether the cell nuclei of various tissues differ in their developmental capacities have been made recently by BRIGGS and KING. These authors developed a technique for the transplantation into frog oocytes of somatic nuclei from embryos of various stages. The recipient oocytes are first enucleated and artificially activated, and the degree of development they reach after nuclear transfer shows the capacity of implanted nuclei to carry the oocyte cytoplasm towards normal differentiation. The above authors have reviewed their investigations recently[1]; whilst blastula nuclei, including nuclei from the dorsal lip of the blastopore, are capable of bringing about the normal development of enucleated oocytes, endoderm nuclei from late gastrula show definite signs of differentiation in that a high percentage of them are incapable of effecting development beyond the gastrula stage.

It has been found difficult to carry out parallel investigations with the oocytes of newts, as these cannot become artificially activated and are normally polyspermic. Attempts made so far[2] involved substantial modifications of the BRIGGS and KING procedure, and the results have not been entirely satisfactory.

It might be of interest to record here a further experiment carried out on *Triturus alpestris* material.

A group of 2 to 4 somatic nuclei were transplanted into a normally fertilized and non-enucleated egg. As a result, this egg carried its normal zygotic nucleus as well as the donor nuclei. It was hoped that the hosts would develop to a more or less advanced stage, at which it would be possible to observe whether the donor nuclei are able to participate in tissues derived from all three cell layers or only in tissues derived from the cell layer of their origin.

To make identification of implanted nuclei possible, haploid embryos were used as donors. These were produced by Dr. G. G. SELMAN of this Institute, who treated *in vitro* newly collected sperm with ultraviolet light of 2100–3200 Å for 1·5 to 3 min. The sperm was then smeared onto oocytes collected from the oviduct. The donors were checked for haploidy by chromosome counts on squashes from a piece of tissue.

The host eggs were left to develop after the nuclear transfer. Sixteen were fixed at the early neural plate stage, sectioned and stained with Feulgen for chromosome counts. The remaining failed to develop normally beyond the early neural plate stage, became abnormal and finally cytolysed.

Examination of the 16 specimens fixed showed that no haploid nuclei could be seen in 5; but several haploid (donor) as well as diploid (host) nuclei were found in each of the remaining 11. Of these 11, 7 had received haploid ectodermal nuclei from the neural plate itself of donor embryos whilst 4 had received nuclei from the chorda mesoderm of the same donors.

It is interesting that, in almost all of the eleven cases, nuclei of donor origin were found in all three cell layers. Thus, not only the embryos as a whole but also each cell layer were nuclear chimaeras, containing nuclei of two distinct origins and chromosome complements, within cytoplasm of one origin.

Sizes of adjacent haploid and diploid cells were markedly different. Incidentally, several tripolar spindles and aneuploid nuclei were also observed.

It follows that at the definite neural plate stage, the nuclei of the mesoderm and the neural plate itself are all capable of organizing cells of any cell layer and are not differentiated at least in this respect. As all hosts failed to complete development, it cannot be decided whether these nuclei differ in their capacity for further tissue differentiation.

In another series, ectodermal nuclei from the neural plate of diploid hosts were transplanted into haploid donors. This series is of less interest than the first one because the nuclei of haploid hosts might be expected in some cases to become diploid and thus be confused with donor nuclei; furthermore, haploid embryos are expected to be more prone to abnormalities. Nine hosts were fixed in this series, 7 as gastrulae and 2 as blastulae. In 3 no reliable chromosome counts could be made. Again, haploid and diploid cells were found in all cell layers of the other four.

As already indicated, the recipients that were not fixed at the early neural plate stage developed abnormalities and cytolysed soon afterwards. It is hoped however that these abnormalities might be avoided or delayed in further tests by: (a) implanting one donor nucleus only instead of a cluster of 2 to 4, and (b) grafting tissues from the chimaeras onto healthy normal embryos, where these tissues might differentiate further.

We wish to thank Professor C. H. WADDINGTON for his interest in this work and Dr. G. G. SELMAN for producing the haploid embryos used. We are thankful to the Melville Trust (E.M.P.) and the Royal Commission for the 1851 Exhibition (J.J.) for Research Fellowships.

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Institute of Animal Genetics, Edinburgh, October 28, 1957.

Zusammenfassung

Gruppen von 2–4 somatischen Kernen haploider *Triturus*-Embryonen wurden in diploide, ungefurchte Eier injiziert. 11 haplo-diploide Mosaik-Larven entwickelten sich bis zum frühen Neurula-Stadium. Nachkommen von Kernen aus dem neuralen Ektoderm oder Chorda-Mesoderm von Spendern im Neurula-Stadium fanden sich in allen drei Keimblättern der Wirtslarve.

The Temporary Inactivation of Newt Larvae by Benzimidazole and its Alkyl Derivatives

Benzimidazole and some of its derivatives, produce muscle relaxation in mammals, apparently by their action on the central nervous system[1]. The experiments

[1] T. J. KING and R. BRIGGS, Cold Spring Harb. Symp. quant. Biol. *21*, 271 (1956).

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[1] L. GOODMAN, A. GILMAN, and N. HART, Fed. Proc. *2*, 80 (1943). – L. GOODMAN and N. HART, Fed. Proc. *3*, 73 (1944). – E. G. DOMINO, K. R. UNNA, and J. KERWIN, J. Pharmacol. *105*, 486 (1952).

reported here show that similar effects are produced in newt larvae by benzimidazole itself and, more effectively, by certain of its alkyl derivatives.

Experimental.–A commercial preparation of benzimidazole was used. The alkyl derivatives were prepared in the laboratory[2].

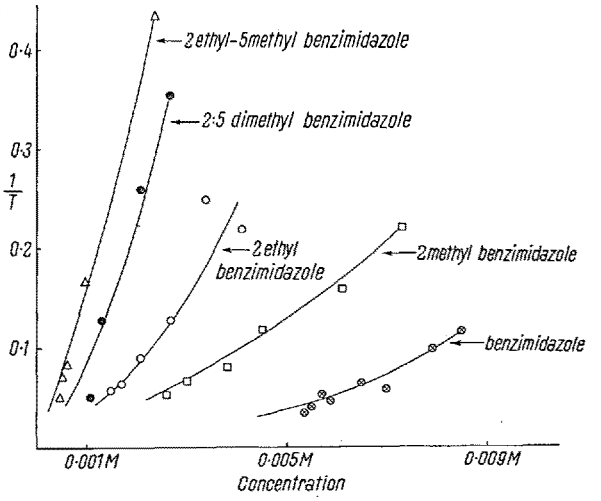
These experiments were performed on larvae of *Triturus alpestris* which had reached a stage of development characterized by the appearance of a third digit on the fore limb. (Comparable with Glaesner's stage 41–42 for *T. vulgaris*[3].) Single larvae were placed in about 10 ml of dilute solutions of benzimidazole and of its 2-methyl, 2-ethyl, 2:5 dimethyl and 2 ethyl-5 methyl derivatives. The solutions were prepared in 1/10th Holtfreter solution and the pH was adjusted, if necessary, to 7·0–7·2 with dilute hydrochloric acid. The concentrations of the benzimidazole used ranged from 70 to 1000 μg/ml.

Results and Discussion.—On placing in the benzimidazole solutions the larvae at first showed increased activity. After a time swimming movements became less frequent, the animals occasionally swimming upside down. Finally the animals became quiescent and no longer responded to contact stimuli. The animals were considered to be completely inactivated when they remained on their backs for at least 2 min after being placed in this position. When returned to 1/10th Holtfreter solution the animals usually responded to contact stimuli within 5 to 10 min, and thereafter behaved normally. The time taken to reach the arbitrary state of inactivation depended on the concentration and nature of the benzimidazole used. After treatment the animals appeared to suffer no ill effects of any kind. Larval development was normal. Most of the animals were alive at least two months after the completion of the experiments.

The results of the experiments are summarized in the Figure. In this graph the reciprocal of the time taken to reach the given inactive state is plotted against the concentration of the benzimidazole employed. In most cases each point represents the mean of four observations. The experiments were performed at a temperature of 20–23°C.

The behaviour of the larvae when placed in the benzimidazole solutions, and their rapid and apparently complete recovery when removed from these solutions, suggests that the compounds exert a depressant action on the nervous system. It is not possible to decide whether the compounds act centrally and produce a muscle relaxation analogous to that seen in mammals, or whether a general narcosis, such as that caused by urethane, is produced.

The results show that newt larvae are more readily inactivated by certain alkyl derivatives of benzimidazole than by benzimidazole itself. The compounds become more effective in order of increasing molecular weight. The alkyl derivatives of benzimidazole are also more effective than the parent compound in producing abnormalities in chick and amphibian embryos[4] and in inhibiting the growth of influenza virus on chick allantois[5]. In particular, in these systems, the 2 ethyl-5 methyl derivative is some 10 to 20 times more potent than the unsubstituted benzimidazole. From the graph, shown in the Figure, it may be calculated that 2 ethyl-5 methyl benzimidazole is about 12 times more active than benzimidazole in depressing the activity of newt larvae.



Alkyl substitution of benzimidazole will give an increased molecular volume, increased basic strength[6], and almost certainly an increased solubility in lipid relative to water. Any of these changes could give rise to the increased potency of the alkyl derivatives. In the case of the 2 ethyl-5 methyl derivative, however, there is a striking coincidence between the increased ionization brought about by alkyl substitution and in the effectiveness of this compound in halting temporarily, or permanently, certain biological processes. Using the method described by ALBERT[7] it may be calculated that at pH 7·0 the 2 ethyl-5 methyl derivative is about 17 times more ionized than is the parent compound. This corresponds, fairly well, to the magnitude of its increased activity, compared with benzimidazole, against diverse biological systems. The evidence suggests that the cationic form of the benzimidazole is essential for these compounds to exert a toxic or narcotic action. This view, however, cannot be completely reconciled with the facts presented by SLONIMSKI[8] which suggest that only the unionized form of benzimidazole is effective in preventing the growth of yeast cells.

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F. BILLETT

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Zusammenfassung

Benzimidazol und einige von seinen Alkylderivaten inaktivieren Molch-Larven schon in kleinen Konzentrationen. In verdünnte Holtfreter-Lösung zurückgebrachte Tiere gewinnen ihre normale Aktivität schnell wieder. Der zeitweilige Beweglichkeitsverlust scheint analog dem bei Säugetieren durch Benzimidazol erzeugten.

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[7] A. ALBERT, *Selective Toxicity* (Methuen, London 1953), p. 206.

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