grafts have been obtained in mongrel dogs which have received a course of four injections of urethane (350 mg/kg) followed by exposure to 900 rad of 250-kv x-rays; in contrast, we have been unable to obtain successful "takes" of homologous bone marrow transplants in dogs which received x-radiation at this supralethal dose (900 rad) without urethane.

The foregoing observations indicate that combined treatment with urethane and x-radiation depresses or inhibits the homograft response in mice and in dogs to a degree considerably greater than that seen with the x-radiation alone. Further, since we have reported previously (4) that the administration of urethane to mice does not depress the capacity of their bone marrow cells to confer protection on otherwise lethally x-irradiated isologous recipients, the present findings suggest a specificity of action of urethane with respect to the cells and tissues (that is, "lymphoid") comprising the immunogenic apparatus. If this is true, it should be possible to inhibit the homograft response for more prolonged periods by administering urethane at suitable intervals after grafting. It also follows from the above that urethane should be useful in the treatment or prevention of the secondary disease syndrome, known to occur after the transplantation of genetically foreign bone marrow cells into lethally x-irradiated recipients. Experiments aimed at these objectives are currently in progress, as well as attempts to induce permanent bone marrow chimerism (see 8) in adult mice by means of homologous marrow cell transplants after treatment with urethane and sublethal doses of x-rays (9).

Note added in proof: Survival of C₃H (H-2k) skin homografts on adult LAF₁ (H-2a, H-2b) recipients for periods beyond 5 months has now been observed in small numbers of LAF₁ mice receiving the following treatments: (i) urethane (given as above) plus 500 rad of x-rays, followed by three intravenous injections of C₃H bone marrow cells and multiple (7) inoculations of specific isoantiserum (anti-LAF₁); (ii) 500 rad plus three intravenous injections of C₃H marrow cells and immunized (anti-LAF₁) C₃H spleen cells.

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References and Notes

1. P. L. T. Ilbery, P. C. Koller, J. F. Loutit, *J. Natl. Cancer Inst.* **20**, 1051 (1958); G. W. Santos, L. J. Cole, P. L. Roan, *Am. J. Physiol.* **194**, 23 (1958).
2. R. Schwartz, J. Stack, W. Damashek, *Proc. Soc. Exptl. Biol. Med.* **99**, 164 (1958).
3. W. R. Meeker, Jr., R. M. Condie, R. A. Good, R. L. Varco, *Ann. N.Y. Acad. Sci.* **87**, 203 (1960).
4. L. J. Cole and S. R. Gospe, *Radiation Research* **11**, 438 (1959); *ibid.* **15**, 684 (1961).
5. D. W. Bailey and B. Usama, *Transplantation Bull.* **7**, 424 (1960).
6. W. E. Davis, Jr., and L. J. Cole, *J. Natl. Cancer Inst.* **27**, 1059 (1961).
7. L. J. Cole and R. M. Garver, *Radiation Research* **12**, 398 (1960).
8. L. J. Cole and W. E. Davis, Jr., *Proc. Natl. Acad. Sci. U.S.* **47**, 594 (1961).
9. These studies have been supported in part by funds from the Bureau of Medicine and Surgery, U.S. Navy Department.

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Chlorpromazine Affects Permeability of Resting Cells of Tetrahymena pyriformis

Abstract. Incubation of cell suspensions of *Tetrahymena pyriformis* with chlorpromazine increased the permeability of the cell membrane. This permeability change could be measured either biochemically by the increased entrance of a chelator or physically by change in light scattering.

During the course of experiments designed to study the mechanism of inhibition of motility of *Tetrahymena pyriformis* by the tranquilizer, chlorpromazine (1), we noticed that one potential reversing agent, L-histidine, increased rather than decreased inhibition. The same amount of L-histidine, in the absence of chlorpromazine, was not toxic.

This observation provided an opportunity to test the idea that the action of chlorpromazine on the intact cell depends, in part, upon ability to alter membrane permeability. If this drug drastically increases permeability, then L-histidine (a normal growth requirement for *T. pyriformis*) might be able to enter the cell in unusually large amounts, and once there, exert its well-known chelation ability. It should then be possible to reverse toxicity induced by histidine and chlorpromazine by the addition of metals. This indeed is the case: the addition of either Ca^{2+}, Fe^{2+}, Mg^{2+} or Zn^{2+}, relieves the inhibition (Table 1).

Confirmation is provided by an experiment in which a nonmetabolized chelator, ethylenediaminetetraacetic acid (EDTA), substitutes for histidine. The same results obtain: the combination of chelator and chlorpromazine is more toxic than chlorpromazine alone (EDTA is not toxic) and the addition of a metal to the chelator-drug combination annuls the toxic effect caused by addition of chelator (Table 1). Besides Fe^{2+} and Zn^{2+}, we also found that Ca^{2+} (0.5 μmole/ml) and Mg^{2+} (0.6 μmole/ml) completely prevented inhibition of motility for the duration of the experiment.

Change in membrane permeability was also demonstrated by the light-scattering techniques that have been used to detect similar changes in mitochondria (2). For these experiments it was unnecessary to add an indicator substance, as increase in membrane permeability was expressed as decrease in light scattering of the treated cell suspension (3).

We conclude that at least part of the action of chlorpromazine in *Tetrahymena*, and probably in other cells as well, depends upon its ability to alter membrane permeability.

As early as 1954, Mann pointed out that chlorpromazine shared with deter-

Table 1. Effect of chelators and metals on the inhibition of motility of *Tetrahymena pyriformis*.

Additions		Percentage* inhibition of motility at elapsed times shown		
Compound	Amount (μmole/ml)	15 min	30 min	45 min
CPZ†	0.15	10	50	80
CPZ + L-histidine‡	7.5	100	100	100
CPZ + L-histidine + Mg^{2+}	0.2	20	40	60
CPZ + L-histidine + Fe^{2+}	0.3	30	70	100
CPZ + L-histidine + Zn^{2+}	0.4	10	20	30
CPZ + L-histidine + Ca^{2+}	0.8	0	10	60
CPZ + EDTA§	1.5	50	90	100
CPZ + EDTA + Fe^{2+}	0.2	0	20	40
CPZ + EDTA + Zn^{2+}	0.35	0	0	10

* These values represent results typical of several experiments.   † CPZ, chlorpromazine. 0.15 μmole/ml was used in all cases.   ‡ 7.5 μmole/ml was used in all cases, since it had no effect upon motility when added singly.   § EDTA, ethylenediaminetetraacetic acid. 1.5 μmole/ml was used in all cases, since it had no effect upon motility when added singly.

gents the ability to cause intracellular sperm proteins to leak into the extracellular medium (4). We suggest that various cells and organ systems be screened for sensitivity of their cell membranes to permeability changes caused by chlorpromazine. The demonstration that tissues vary in ability to absorb this drug (5) provides a clue to which cell membranes may prove to be sensitive. If differential effects are shown, chlorpromazine may be used in combination therapy to increase accessibility of cell interiors to the second drug, and may be used as a laboratory tool for increasing the range of compounds which can reach the interior of the cell without resorting to complete cell breakage.

That cells can be made "leaky" without impairing their ability to reproduce has recently been shown (6). Preliminary data indicate that the site of action of chlorpromazine is a lipid. Cytochemistry and electron micrography will be required for independent confirmation.

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References and Notes

1. Experimental methods followed the general form of those described by M. Sanders and H. A. Nathan, *J. Gen. Microbiol.* **21**, 264, (1959). One simplification of technique substituted a growth medium composed of proteose-peptone (1.0 percent) plus glucose (1.0 percent) for the more expensive defined medium used earlier. It should be noted that organisms taken from cultures of different ages differ in sensitivity to chlorpromazine. Thus to obtain repeatable results it is necessary to fix rigid methods for cultivation of *Tetrahymena* with respect to physiological age of culture and conditions of cultivation such as temperature and amount of aeration.
2. L. Packer, *J. Biol. Chem.* **236**, 214 (1961).
3. We thank L. Packer, University of Texas, Southwestern Medical School, for performing this experiment.
4. T. Mann, *Proc. Study Fertility* No. 6, 41 (1954).
5. M. Berger, *J. Neurochem.* **2**, 30 (1957).
6. H. A. Nathan, *Nature* **192**, 471 (1961).

31 October 1961

Perseveration Factor

In their report, "Cerebral dysfunction and intellectual impairment in old age" [*Science* **134**, 1518 (1961)], Misiak and Loranger present a centroid factor analysis of seven tests showing a clear two-factor structure and then dismiss it with the statement, "The analysis yielded only one significant factor, a general intellectual one in which both critical flicker frequency and age have signifi-

Table 1. Results of an oblique rotation of the centroid factor matrix from the analysis of Misiak and Loranger.

Variables	Factor A	Factor B
1. Critical flicker frequency	0.56	−0.07
2. Digit Symbol	.30	.48
3. Porteus Maze	.23	.38
4. PMA Reasoning (untimed)	.00	.72
5. Raven Progressive Matrices	.00	.71
6. WCST, perseverative errors, reversed*	.64	.08
7. Age, reversed†	.33	.08

* Freedom from perseverative errors.
† "Youngness."

cant loadings." By the Bargmann-Bartlett test, the probability that one factor is sufficient is .029, while the probability that two are sufficient is .935, so actually both of their centroid factors are significant.

An oblique rotation of their centroid factor matrix yields the pattern given in Table 1. In making this rotation we first reversed their 6th and 7th variables (WCST, perseverative errors, and age). Then lines ("hyperplanes") were passed through the centroids of variables 4 and 5 and 1 and 6. The factor pattern given in Table 1 shows the projections on the corresponding reference vectors.

Misiak and Loranger state that "it is tempting to draw similarities between conceptual perseveration and the neurophysiological perseveration reflected in flicker-fusion. However, the failure of the factor analysis to uncover a perseverative factor somewhat inhibits such speculation." The conclusion they wanted to draw but didn't is entirely justified by their data. All they needed to do to uncover a perseveration factor was to rotate their centroid matrix, as we have done in Table 1, to approximate simple structure. Factor *A* (Table 1) is a lack-of-perseveration factor, with high loadings on variables 1 and 6; intermediate loadings on 2, 3, and 7; and zero loadings on 4 and 5. Factor *B* is a reasoning factor, with high loadings on variables 4 and 5; intermediate loadings on 2 and 3; and near-zero loadings on 1, 6, and 7.

The cosine of the angle between the reference vectors is −0.50, indicating that the two factors are positively but not highly correlated.

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There appears to be no single infallible criterion of when a proper number of factors have been extracted. At least 25 different criteria have been proposed. In the present problem we employed Humphrey's rule [B. Fruchter, *Introduction to Factor Analysis* (Van Nostrand, New York, 1954)]. Computationally this is one of the more facile solutions, and correspondingly perhaps one of the cruder ones. The rule of thumb is that if the product of the two highest loadings in a column of the centroid factor does not exceed twice the standard error of a correlation coefficient of zero, the factor is probably not significant. In our problem the product was 1.29 times the standard error.

We are indebted to Cureton and his associates for applying to our data Bargmann's improved matrix formulation of the Bartlett test, a considerably more exact solution than that which we employed. The Bargmann-Bartlett test ordinarily is feasible only with an electronic computer. However, with our small seven-variable matrix, hand computation is quite practicable, as Cureton *et al.* have demonstrated.

Of course the factorial study was a subsidiary analysis. The principal objective was to relate intellectual ability in old age to critical flicker frequency. We were also looking for a perseverative factor, and the analysis by Cureton *et al.* uncovered one which had escaped our analysis. The discovery in the elderly of an apparent neurophysiological perseveration associated with conceptual perseveration is most interesting. However, we do feel that this finding is subject to confirmation with a larger battery of tests of intellectual ability than we employed.

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Visual System at Fusion

An error in our report entitled "Nonlinear property of the visual system at fusion" [*Science* **134**, 612 (1961)] has been communicated to us by J. Levinson (Bell Telephone Laboratories, Mur-