Reports and Letters

Antagonism of 5-Hydroxytryptamine by Chlorpromazine

In the course of studies of the mechanism of the vascular injury induced in rats by a group of agents that damage mast cells and liberate histamine (ovomucoid, 48/80, dextran, and testis extract), it was discovered that 5-hydroxytryptamine as well as histamine is capable of producing hyperemia and edema when injected subcutaneously into rats (*1*). It was also found that 5-hydroxytryptamine or a related substance is associated with mast cells and is liberated along with histamine by the "histamine liberators" (*2*). It has been demonstrated that dibenamine inhibits the *in vitro* action of 5-hydroxytryptamine on the rat colon (*3*). We therefore tested it *in vivo* as an antagonist of the edematous response to 5-hydroxytryptamine and found it a potent inhibitor. It is known that chlorpromazine has antihistaminic activity (*4*) and adrenergic blocking properties similar to those of dibenamine (*5*). Consequently, we examined chlorpromazine for its anti-5-hydroxytryptamine properties and antihistaminic properties *in vivo* in the rat and *in vitro* on the rat colon and found it to be a potent 5-hydroxytryptamine antagonist as well as an antihistaminic (*6*).

Female albino rats (Sprague-Dawley) weighing 160 to 200 g were used in these experiments. Subcutaneous injections of the various agents in solution in freshly made 0.86-percent NaCl were given in the dorsal skin of the paws of rats. Saline was used for control injections. The subcutaneous injections were made by carefully inserting a 27-gage needle between the third and fourth digits of the paws to about the midpoint of the dorsum of the paw; 0.05 ml was injected in the fore paw and 0.10 ml in the hind paw. Each side of the rat, fore and hind paw, was used to measure the action of a single edema-producing agent; two agents were thus tested simultaneously in each rat. Four or more rats were used to test each dose of an agent, and the injections were rotated from rat to rat so that the right and left sides were used an equal number of times for each agent. Evans blue, 0.5 ml of a 0.4-percent solution in 0.86-percent saline, was injected via the tail vein immediately prior to the local injection. Intense bluing of the skin is an evidence of leakage of plasma protein because the dye is bound to the plasma protein. There was little or no bluing of saline-treated paws. The following concentrations of the agents were used: 5-hydroxytryptamine (*7*), 1 μg/ml; and histamine, 200 μg/ml (each as the free base). These concentrations of the edema-producing agents gave approximately the same response as measured either by the increase in tissue water content or by the grossly evident swelling and bluing of the skin (*2*). The local vascular response was graded grossly from 0 to 4+, 2 hours after local injection; at this time the induced edema was still maximal and the swelling from saline injection had largely disappeared. The agreement between the gross observations and the measurements of edema by increase in tissue water, estimated by removal of the skin and drying to constant weight at 100°C for 5 days, was excellent.

Chlorpromazine (*8*) was tested in doses of 0.5 to 3.0 mg/kg of body weight. The drug was administered in 0.86-percent saline solution via the tail vein 15 minutes prior to the local injection of histamine or 5-hydroxytryptamine. With a dose of chlorpromazine of 0.5 mg/kg, little decrease in vascular injury was observed. At doses of 1.0 and 1.5 mg/kg, the action of both histamine and 5-hydroxytryptamine was completely abolished. The 3.0 mg/kg dose produced an observable lethargy, convulsions, and occasional death in the animals.

Chlorpromazine was also tested on the rat colon as antagonist to 5-hydroxytryptamine and acetylcholine. The method used was that of Dalgliesh, Toh, and Work (*9*). Atropine was omitted from the bath fluid. The bath volume was 16 ml. For the test, the strip was standardized to give about one-half maximal contraction to each of the stimulants. For 5-hydroxytryptamine, the quantity was 0.025 μg and for acetylcholine it was 0.05 μg. Exposure of the strip to 10 μg of chlorpromazine for 3 minutes inhibited the reaction to both stimulants by 50 per cent or more. Addition of 20 μg of chlorpromazine to the bath reduced the activity to almost nothing. Complete recovery of activity then required about 30 minutes. Essentially the same result was obtained with strips from 3 rats.

The full range of the specific pharmacological antagonisms of chlorpromazine has not been worked out. The known activities have recently been summarized (*10*). They are mild histamine, acetylcholine, noradrenalin antagonism and powerful adrenalin inhibition. To this we now add the 5-hydroxytryptamine antagonism (*11*). This action is of considerable interest in terms of the rapidly appearing evidence of the importance of this agent as a neurohumor (*12*). Chlorpromazine has been used with good effect in a variety of conditions including mental disease (*13*), nausea and vomiting (*14*), and alleviation of pain (*15*). We suggest that the demonstrated antagonism of chlorpromazine to 5-hydroxytryptamine may contribute to an understanding of the diverse actions of the drug and that the antagonism merits further investigation.

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References and Notes

1. E. P. Benditt, S. Bader, K. S. Lam, *Arch. Pathol.* 60, 104 (1955).
2. D. A. Rowley and E. P. Benditt, in preparation.
3. E. Fingl and J. H. Gaddum, *Federation Proc.* 12, 320 (1953).
4. J. Kopera and A. K. Armitage, *Brit. J. Pharmacol.* 9, 392 (1954).
5. F. Huidobro, *Arch. intern. pharmacodynamie* 98, 308 (1954).
6. This work was supported in part by grants from the American Heart Association and grant H-1073 from the National Heart Institute of the U.S. Public Health Service.
7. 5-Hydroxytryptamine creatinine sulfate obtained courtesy of George Berryman, Abbott Laboratories, North Chicago, Ill.
8. Thorazine, Smith, Kline and French Laboratories, Philadelphia, Pa.
9. C. E. Dalgliesh, C. C. Toh, T. S. Work, *J. Physiol. London* 120, 298 (1953).
10. *Lancet* 268, 337 (1955).
11. Since this report was submitted, it has come to our attention that the antagonism of chlorpromazine to 5-hydroxytryptamine, with the nictitating membrane of the cat as the test object, has been described by J. Lecomte, *Arch. intern. pharmacodynamic* 100, 457 (1955).
12. I. H. Page, *Physiol. Revs.* 34, 563 (1954); A. H. Amin, T. B. B. Crawford, J. H. Gaddum, *J. Physiol. London* 126, 596 (1954).
13. H. E. Lehmann and G. E. Hanrahan, *Arch. Neurol. Psychiat.* 71, 227 (1954).
14. E. M. Boyd, C. E. Boyd, W. A. Cassell, *Can. Med. Assoc. J.* 70, 276 (1954).
15. J. Cahn, M. Dubrasquet, M. J. Melon, *Anesthésie et analgésie* 10, 89 (1953).

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Method for Distinguishing Intact Cells from Free Nuclei

In recent communications, Brown (*1*) and Allfrey and Mirsky (*2*) have stressed the lack of any convenient and reliable method for distinguishing whole cells from free nuclei, particularly in the case of small lymphocytes with very scanty cytoplasm. We wish to draw attention to a simple method that has given promising results with many types of material that we have examined. This is based on

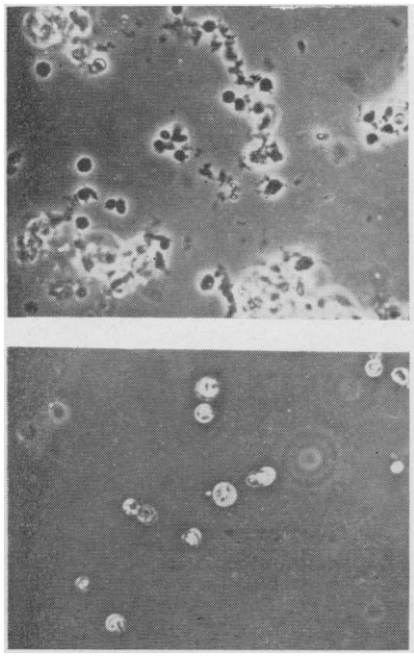

Fig. 1. (Top) Field showing free nuclei of mouse spleen immersed in a 28-percent protein solution; (bottom) field showing intact cells in the same medium (×300).

a method of cell refractometry that has been described elsewhere (*3*).

When living cells are immersed in an isotonic protein medium having a refractive index greater than that of the cytoplasm, the latter appears bright instead of dark by positive phase contrast. This depends on the impermeability of normal cytoplasm to proteins. If the cell is killed by fixation or other drastic chemical methods, the cytoplasm becomes permeable to proteins and remains dark. Mild physical damage or autolytic changes result in cytoplasmic swelling and bleb formation but do not usually affect the impermeability to proteins; as the refractive index decreases with swelling, the bright appearance is enhanced. Free nuclei, on the other hand, appear to be permeable to proteins and always remain dark even in highly concentrated protein solutions. The cytoplasm of most normal tissue cells has a refractive index of less than 1.370, which corresponds to a protein concentration of about 20 percent. In order to avoid excessive dilution of protein by the suspension medium it is convenient to make up a stock solution containing about 30 percent protein. Armour's bovine plasma albumin fraction V has been used for this purpose.

The adjustment of tonicity is important (*3*), but for mammalian tissues it is sufficient to make up a 30-percent protein solution in 0.6-percent sodium chloride. A small droplet of homogenate suspension is mixed on a slide with a large drop of protein and then examined by phase-contrast or interference microscopy. Figures 1 and 2 illustrate the technique as applied to preparations from the spleens of 7-day old mice.

The method used to prepare free nuclei (*4*) depends on the use of a medium containing 0.12*M* KCl with small amounts of NaCl and $CaCl_2$ and 6 percent bovine plasma albumin, brought to *p*H 7.1. A glass homogenizer of the Potter-Elvejhem type was used to fragment the tissue. Several methods involving the use of sucrose have also been tried, but they appear to give less satisfactory results and smaller yields.

In Fig. 1, the top photograph is a low-power view showing a field containing free nuclei in a 28-percent protein medium, and the bottom photograph shows a similar field containing intact cells.

Figures 2 and 3 show a number of higher-power views taken of a less vigorously treated preparation containing a mixture of free nuclei and intact cells. On the left in Fig. 2 is a single intact cell and a group of three free nuclei with a little granular debris. The nuclei are surrounded by the well known phase-contrast halo, but there is no bright zone of cytoplasm. The bright cells are actually surrounded by a dark halo that is more easily seen under the microscope than in the photographs. A small lymphocyte and a free nucleus are shown on the right in Fig. 2. The very narrow zone of bright cytoplasm is clearly visible under the microscope and cannot be confused with a naked nucleus. Figure 3 illustrates another interesting point: the intact cells frequently float at a slightly different level from the free nuclei. In the top photograph, an intact cell has been focused and the free nucleus appears out of focus.

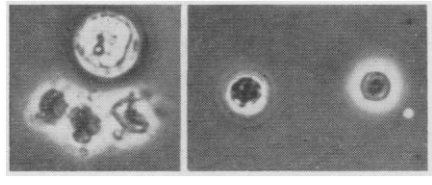

Fig. 2. (Left) Intact cell and three isolated nuclei; (right) small lymphocyte with very narrow ring of bright cytoplasm and naked nucleus. Both photographs taken in 28-percent protein medium (×700).

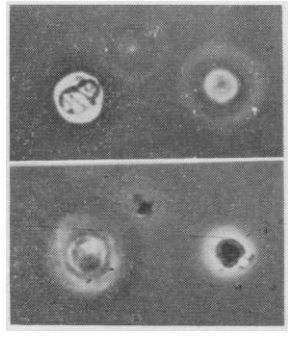

Fig. 3. (Top) Intact cell in focus, nucleus out of focus; (bottom) nucleus in focus, intact cell out of focus. These photographs illustrate the fact that cells and nuclei usually float at different levels. Both photographs taken in 28-percent protein medium (×700).

In the bottom photograph, the position has been reversed and the free nucleus is in focus. A tiny bleb of bright cytoplasm can be seen adhering to the nucleus. Such blebs usually disappear in the course of time.

We believe that this simple test may prove to be of value to other workers in this field (*5*).

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References and Notes

1. J. R. C. Brown, *Science* 121, 511 (1955).
2. V. G. Allfrey and A. E. Mirsky, *ibid.* 121, 879 (1955).
3. R. Barer, K. F. A. Ross, S. Tkaczyk, *Nature* 171, 720 (1953); R. Barer and S. Joseph, *Quart. J. Microscop. Sci.* 95, 399 (1954), 96, 1 (1955), and in press; R. Barer, *Naturwissenschaften* 41, 206 (1954).
4. A detailed report of the method is in preparation.
5. This work was supported by the Rockefeller Foundation and by the Medical Research Council of Great Britain.

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Leap Year and Calendar Reform

John J. Case has called attention [*Science* **122**, 648 (7 Oct. 1955)] to a kind of calendar reform that has been largely overlooked by calendar reformers, the returning of the zero point in the calendar year to the solstice, an appropriate point in the seasonal year. He suggests that this could be accomplished by dropping out 10 days, doing again what Pope Gregory did in 1582. The same good end could be achieved by a much less drastic operation; we have all the apparatus we need in our control of the leap year rule. Let us simply agree to do without leap years until the calendar rights itself—that is, no more this century after 1956. Then the year A.D. 2000 would be a very propitious time to begin the use of leap years again.

The adoption of this proposal would not disturb our accepted 7-day week, which is the point of greatest resistance to most proposals of calendar reform. It would even simplify slightly comparative business records for some time to come. And if, within a generation or so, we do venture a major calendar reform involving weeks and months, we should be able to get the new one smoothly running for a while without the added complication of an extra leap year day outside the weekly sequence.

When we resume leap years in A.D. 2000, we would do well to substitute a