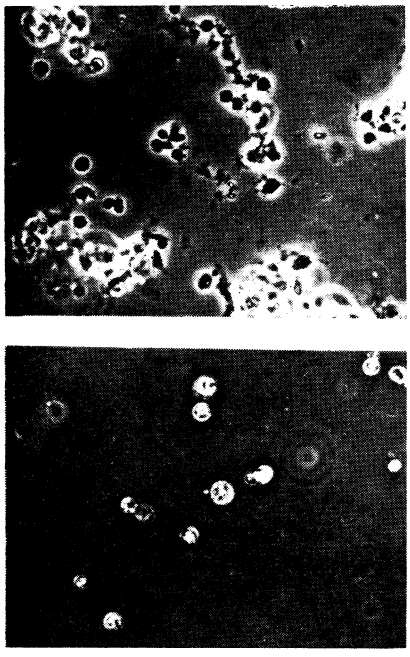

Fig. 1. (Top) Field showing free nuclei of mouse spleen immersed in a 28-percent protein solution; (bottom) field showing intact cells in the same medium (×300).

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.

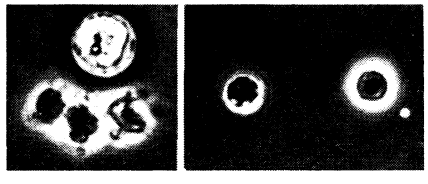

Fig. 2. (Left) Intact cell and three isolated nuclei; (right) small lymphocyte with very narrow ring of bright cytoplasm and naked nucleus. Both photographs taken in 28-percent protein medium (×700).

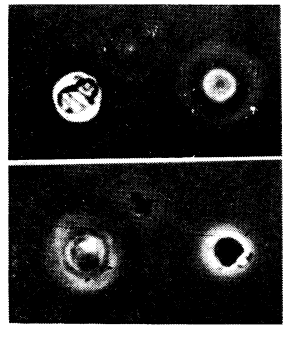

Fig. 3. (Top) Intact cell in focus, nucleus out of focus; (bottom) nucleus in focus, intact cell out of focus. These photographs illustrate the fact that cells and nuclei usually float at different levels. Both photographs taken in 28-percent protein medium (×700).

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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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.

1 August 1955

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

better leap year rule for our present Gregorian rule, which is neither simple nor as accurate as it should be. Our present rule makes the average year 365 97/400 days. The actual tropical year is shorter than this, and it is growing steadily shorter by a long-term slow change. The most accurate simple leap year rule just now would be the following: a leap year every 4th year unless the year number is divisible by 128 (this gives an average year that was a perfect fit in 1910). To keep in step for thousands of years ahead, we would prefer a simpler rule giving us a leap year every fourth year unless the year number is divisible by 120. With this rule, there would be no appreciable drift over the next 8000 years. Indeed, the maximum drift up to the year A.D. 10,000 would be scarcely more than the range of drift that is inescapable within any 4-year cycle according to any kind whatever of leap year rule. By comparison, the continued use of our Gregorian leap year rule would shift the calendar more than a week by A.D. 10,000. Besides, the improved rule—every 4th year a leap year unless the year number is divisible by 120—would be easier to remember and easier to apply. Having once gotten our calendar into step with the astronomical tropical year, this rule would keep us in step for a very long time to come.

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7 November 1955

Effect of Ergot Drugs on Betta splendens

It has been reported in a previous communication (*1*) that the Siamese fighting fish, *Betta splendens,* responds to low concentrations of *d*-lysergic acid diethylamide (LSD-25) with a quiescent state that is typified by at least nine easily observable changes in the vegetative, motor, and behavioral characteristics of the fish. This communication summarizes an attempt to determine which, if any, of these nine changes are, indeed, specific for this drug (*2*).

The method employed consisted of exposing groups of three fish to equimolecular solutions of LSD-25 and of eight other ergot derivatives that included two optical isomers of LSD-25 (*l*-LSD-25 and *d*-isoLSD-25), a monobromo derivative of LSD-25 (BOL-148), *d*-lysergic acid, *d*-lysergic acid ethylamide (LAE-32), ergotamine, dihydroergotamine, and ergonovine. Mescaline and meperidine hydrochloride (Demerol) were also tested. The fish were observed continuously over a period of 4 hours; after this they were washed, transferred to fresh spring water, and observed at longer intervals.

Table 1. Response of *Betta splendens* to ergot drugs, mescaline, and Demerol.

Response	LSD-25	*l*-LSD-25	*d*-Iso-LSD-25	BOL-148	LAE-32	*d*-Lysergic acid	Ergonovine	Ergotamine	Dihydroergotamine	Mescaline	Demerol
Backward movement with pectoral fins	x			x	x						
Head up at surface	x			Atypical	Atypical, usually at bottom						x
Cartesian diver (vertical)	x			Rare	x						
Barrel-roll (vertical)	x			Rare	Rare						
Body kinking	x			Rare	Rare						
Quiescent state	x			x	x						
Slow deliberate movements	x			x	x	x		x			
Lateral display	x	x	x	x	x	x	x	x		x	
Darkened pigment	x			x	x				x		

Experiments were performed at concentrations varying from $5 \times 10^{-7}M$ (approximately 0.2 μg of LSD-25 per milliliter) to $5 \times 10^{-5}M$ for the ergot drugs. LSD-25 was active over the complete range. Mescaline and Demerol were completely inactive at this level and were run at concentrations of 2.5 mg/ml and 0.6 mg/ml, respectively, the highest levels of these drugs that are not rapidly lethal. Table 1 reports the results of an experiment at the $5 \times 10^{-5}M$ level. It can be seen that, even at relatively high concentrations, the first five criteria—which seem to define a syndrome of loss of control of the musculature of the trunk that is possibly accompanied by a derangement of hydrostatic bladder function and the quiescent state—are sufficient to differentiate LSD-25 from the other drugs in the table. Both LAE-32 and BOL-148 resemble LSD-25 relatively closely, but both rarely induce the spastic kinking produced by LSD-25 that causes the fish to look like commas when they are viewed from the side and often like letter *s*'s when they are viewed from above. Neither do LAE-32 and BOL-148 cause the fish to assume an almost vertical position near the surface of the water for long periods of time. The symptoms induced by LAE-32 appear later than those induced by either BOL-148 or LSD-25. Fish exposed to BOL-148 in concentrations above 5 μg/ml often die; concentrations of the order of 0.5 μg/ml show no effect beside an increase in pigmentation and a decrease in activity. Since the induction of the torpor varies inversely with the dosage and may take an hour to develop at dosages of the order of 1 μg/ml, there is a possibility of mistaking the BOL-148 reaction for a response to LSD-25 at this concentration if the fish are not observed long enough.

As previously stated, the fish may become essentially quiescent for days. Arousal occurs at the slightest stimulus but is followed by an immediate return to relative inactivity. During the period of entrance into the quiescent state and during the period of emergence therefrom, the fish have been observed to show their typical rage reaction to other fish. The rage reaction lasts only a few seconds, but the full expansion of dorsal, ventral, and (in the case of LSD-25) pelvic fins frequently occurs even though the fish are otherwise essentially quiescent.

The specific effect on *B. splendens* of the diethyl amide group of LSD-25 is therefore vulnerable to changes in either spatial or chemical variations in the structure of LSD-25. Similarly, in man only LSD-25 shows its special effects (*3*). However, the action on brain metabolism as measured by oxygen consumption does not depend on this spatial specificity but on the chemical structure (*4*).

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References and Notes

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2. This investigation was supported by grants from the Josiah Macy, Jr., Foundation and the Geschickter Fund for Medical Research, Inc. We are indebted to Sandoz Chemical Works, Inc. for supplies of the lysergic acid diethylamide and its derivatives.
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3 August 1955

Formation of Radioactive Protein-Bound Monoiodotyrosine by Stored Thyroid Slices

During the course of our investigations of thyroidal iodine metabolism, the observation was made that cattle thyroid slices or lobes that have been stored in the refrigerator (or deep-freeze unit) for 24 hours or more retain much of their ability to form protein-bound I^{131} from iodide-I^{131} present in the Krebs-Ringer bicarbonate buffer incubation medium. We have now investigated the I^{131} metabolism of stored thyroid slices (*1*) in some