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.

GEORGE W. WALKER
Walden Presbyterian Church
2065 Bailey Avenue, Buffalo, New York

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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*).

L. T. EVANS
L. H. GERONIMUS
C. KORNETSKY
H. A. ABRAMSON
Biological Laboratory,
Cold Spring Harbor, New York

References and Notes

1. H. A. Abramson and L. T. Evans, *Science* 120, 990 (1954).
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.
3. M. Jarvik, H. A. Abramson, M. Hirsch, *J. Abnormal Social Psychol.*, in press.
4. L. H. Geronimus, H. A. Abramson, L. J. Ingraham, in preparation.

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

detail and find that, in contrast with surviving (fresh) thyroid slices (*2*), stored thyroid loses ability to (i) form diiodotyrosine, (ii) synthesize thyroxin, and (iii) concentrate iodide from the incubation medium. Stored thyroid slices, however, do retain an ability to form protein-bound monoiodotyrosine and are considerably more productive in this respect than the copper-fortified thyroid homogenates that have recently been shown to be capable of producing monoiodotyrosine (*3, 4*).

The afore-mentioned deficiency of stored thyroid slices with respect to the ability to concentrate inorganic I^{131} was demonstrated when stored slices were incubated in media containing thiouracil, sodium *p*-aminosalicylate (PAS), or Tapazole—all of which inhibit organic binding of iodine in the thyroid. The data in Table 1 show that stored slices accumulated much less inorganic I^{131} than fresh thyroid slices. Incubations were carried out in quadruplicate for 3 hours by the procedure of Morton and Chaikoff (*2*); standard deviations are tabulated. The measurements summarized in Table 2 further indicate that stored slices lose ability to concentrate inorganic I^{131}. Over a 13-fold variation in the ratio of the weight of the slices to the weight of the medium and slices, the ratio of the inorganic I^{131} in the slices to that in the medium and slices changed correspondingly; and the quantity of inorganic I^{131} in the slices after incubation was never more than would diffuse into an equal weight of inert aqueous material.

The composition of the protein-bound I^{131} was established by paper chromatography, with collidine-H_2O-NH_3 development (*5*), of stored slices hydrolyzed (after incubation in the I^{131} medium) with pancreatin (*5*). Forty microliters of the pancreatic hydrolyzate were placed along a 4 cm line (origin) on Whatman No. 1 filter paper. The autoradiographs of Fig. 1 show that little if any diiodotyrosine (DIT) or thyroxin (TX) was formed and that the predominant component of the protein-bound I^{131} was monoiodotyrosine (MIT). A high R_f component similar to that found in thyroid homogenate (*4, 5*) was also present in the incubated, stored slices.

Formation of MIT by stored slices is apparently enzyme-dependent since a 1-minute boiling of the slices before incubation decreased MIT production by a factor of 30, and adding $10^{-4}M$ Cu^{++} to the medium did not restore MIT production (Table 3). The addition of 10^{-3} or $10^{-4}M$ Cu^{++} or Co^{++} to the incubation medium did not markedly alter MIT formation in stored, unboiled slices. Thiocyanate, at $10^{-3}M$, inhibited organic binding of I^{131} by stored thyroid. Incubation at 25°C in air (ordinarily, incubation of thyroid slices is carried out at 37.5°C in an atmosphere of 95 percent oxygen and 5 percent carbon dioxide) did not decrease MIT formation, although reducing incubation temperature to 4°C did diminish the rate of MIT production. Incubation in the presence of $10^{-3}M$ KCN or in a nitrogen atmosphere did not depress MIT formation. Homogenizing stored slices before incubation reduced protein-bound I^{131} production by a factor of 6—to about the level found in studies with thyroid homogenates (*3–5*).

From the time of the identification of monoiodotyrosine as a natural constituent of the thyroid gland (*6*), MIT was thought to represent the first step in the formation of DIT (*7*). The results of this study show, however, that a relatively large proportion of MIT can be produced with the formation of only minimal quantities of DIT. Further, the observations that MIT is produced when incubation takes place in the presence of

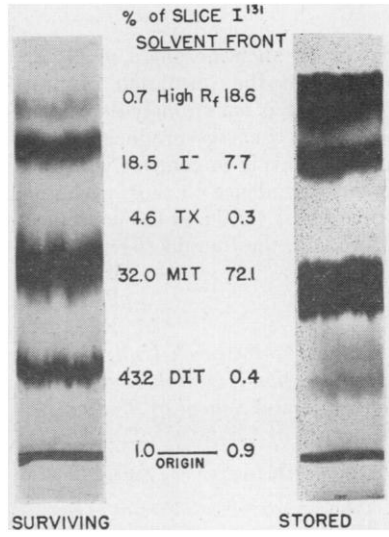

Fig. 1. Autoradiographs of chromatograms from surviving and stored thyroid slices incubated in I^{131} medium.

Table 1. Comparison of surviving and stored thyroid slices.*

Compound	I^{131} in slices, as percentage of total I^{131} in medium plus slices			
	Surviving		Stored	
	Protein-bound I^{131}	Inorganic I^{131}	Protein-bound I^{131}	Inorganic I^{131}
Controls	51.1 ± 3.8	12.1 ± 1.0	36.9 ± 3.0	4.1 ± 0.3
$10^{-3}M$ thiouracil	2.8 ± 0.2	21.6 ± 2.0	0.8 ± 0.1	3.5 ± 0.2
$10^{-4}M$ thiouracil	6.2 ± 0.4	19.7 ± 1.4	1.0 ± 0.1	4.4 ± 0.4
$10^{-3}M$ PAS	3.2 ± 0.1	19.9 ± 1.6	1.5 ± 0.1	3.6 ± 0.1
$10^{-4}M$ PAS	9.6 ± 0.3	25.8 ± 1.1	3.2 ± 0.1	4.5 ± 0.4
$10^{-4}M$ Tapazole	5.0 ± 0.4	20.3 ± 0.9	1.1 ± 0.1	3.8 ± 0.3

* All stored thyroid slices employed in the measurements for Tables 1, 2 and 3 were stored at − 16°C for 2 weeks before incubation.

Table 2. Effect of varying ratio of slice weight to medium volume.

Slice weight (mg)	Medium volume (ml)	wt. of slices/ wt. of medium and slices	I^{131} of slices/ I^{131} of medium and slices
150 mg slices,	3.0 ml medium	1/21	1/24 ± 2
150 mg slices,	12.0 ml medium	1/81	1/99 ± 8
600 mg slices,	3.0 ml medium	1/6	1/7.3 ± 0.6

Table 3. Factors affecting stored thyroid metabolism.

Factor	I^{131} in slices, as percentage of total I^{131} in medium plus slices		
	Total I^{131} in slices	Protein-bound I^{131}	Inorganic I^{131}
Controls	41.1 ± 2.9	36.9 ± 3.0	4.1 ± 0.3
Boiled 1 min	4.0 ± 0.1	1.1 ± 0.1	2.8 ± 0.1
Boiled + $10^{-4}M$ Cu^{++}	4.2 ± 0.2	1.3 ± 0.1	2.8 ± 0.1
$10^{-4}M$ Cu^{++}	40.6 ± 2.5	36.8 ± 2.7	3.9 ± 0.3
$10^{-3}M$ KSCN	5.3 ± 0.4	3.2 ± 0.3	2.1 ± 0.3
$10^{-3}M$ KCN	40.2 ± 3.7	35.0 ± 3.1	4.3 ± 0.3
25°C in Air	42.6 ± 3.8	38.4 ± 3.2	4.1 ± 0.4
N_2 Atmosphere	41.9 ± 3.2	37.2 ± 2.8	4.2 ± 0.4
4°C	8.2 ± 0.7	6.1 ± 0.5	2.3 ± 0.2
Homogenized	7.0 ± 0.5	6.3 ± 0.4	0.7 ± 0.1

CN^- or in an atmosphere of N_2 or air suggest that the formation of protein-bound MIT is not strongly dependent on the oxidative, energy-producing cycles of the cell. This is in contrast with the apparent dependence on those cycles of the formation of DIT and the concentration of iodide in the thyroid (*8*).

S. W. MAYER
F. H. KELLY
M. E. MORTON*
Radioisotope Research Unit, Veterans Administration Hospital, Long Beach, California and *School of Medicine, University of California, Los Angeles*

References and Notes

1. This work was supported in part by grant A-798 from the National Institutes of Health, U.S. Public Health Service.
2. M. E. Morton and I. L. Chaikoff, *J. Biol. Chem.* 147, 1 (1943).
3. D. M. Fawcett and S. Kirkwood, *J. Biol. Chem.* 205, 795 (1953).
4. J. B. Wyngaarden and J. B. Stanbury, *J. Biol. Chem.* 212, 151 (1955).
5. A. Taurog, G. D. Potter, I. L. Chaikoff, *J. Biol. Chem.* 213, 119 (1955).
6. K. Fink and R. M. Fink, *Science* 108, 358 (1948).
7. J. Gross and R. Pitt-Rivers, *Vitamins and Hormones*, vol. 2 (Academic Press, Inc., New York, 1953), p. 163.
8. N. Freinkel and S. H. Ingbar, *J. Clin. End. Metab.* 15, 442 (1955).

* Present address: 117 E. 8 St., Long Beach, Calif.

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Protection of Mouse Fetus against X-irradiation Death

The value of cysteinamine (beta-mercaptoethylamine) as a protective agent against ionizing radiations in the adult mammal was first established by Bacq (*1*) and has been confirmed by other investigators. In our laboratory, a study by Rugh and Wang (*2*) indicated that the effect of 700-r x-rays, the minimum $LD_{100/30}$ for CF_1 adult male mice, is modified by the intraperitoneal injection of 3 mg of cysteinamine 5 to 30 minutes before irradiation so that only 30-percent mortality occurs within the specified period. It was thought to be important to determine whether the protective value of this –SH compound could be transferred to the mammalian fetus that is x-irradiated *in utero* by the injection of the drug into the pregnant animal. Although the radiosensitivity of the fetus has been extensively investigated in terms of developmental abnormalities and lethality (*3*), no such protection studies on the fetus have been reported in the literature.

The animals used in this study (*4*) were CF_1 female mice that were x-irradiated on gestation days 13.5 to 19.5. Doses ranged from 300 to 1200 r, delivered at a rate of 96.5 r/min air dose as measured at the level of the gravid uterus. The x-ray facilities consisted of a Quadrocondex constant-potential therapy machine run at 210 kvp peak, 15 ma, and a distance of 50 cm from the target to the animal, with 0.28 mm Cu and 0.50 mm Al filters added. Cysteinamine was made up in physiological saline at a concentration of 3 mg/ml, 1 ml of which was administered intraperitoneally to the pregnant mouse 20 minutes before exposure to x-rays.

Each experiment consisted of three groups of mice. One control group received cysteinamine without irradiation. A second control series received x-irradiation alone; the third or experimental group of mice received both the drug and x-irradiation. After delivery of the offspring, the litters were counted daily and weighed at weekly intervals for 1 month. In cases in which the mice received a lethal dose of irradiation, litters were exchanged at birth with simultaneously dropped control litters in order that death or possible retardation of lactation in the irradiated mothers might not affect the growth and viability of the offspring. Data from cysteinamine-injected controls have in no way differed from uninjected control values.

Considering the weight at age 1 month, although the irradiated mice show an average weight difference of almost 5 g less than that of the non-irradiated controls, prior administration of the drug allows average weight very nearly equal to that of the controls (Table 1).

In Table 2 appear data from an experiment in which an exchange was made between treated litters and mothers and noninjected controls. Both irradiated groups showed a decreased birth weight below control weights with but slight difference between the two treated groups. All fetuses irradiated without cysteinamine died within the first 10 days after delivery, while those that had received prior cysteinamine injections exhibited 78.6 percent survival (protection to 1 month).

It seems reasonable to hypothesize that a reduction in litter size, particularly in the noncysteinamine-injected but irradiated groups, could be the result of their greater susceptibility to death *in utero*. In the data obtained following irradiation at 14.5 days of gestation (Table 1), the percentage values might seem to suggest a range of sensitivity favoring the cysteinamine-injected mice. However, the control and drug-injected groups are quite similar in weight, and an apparently significant weight difference exists between these two groups and those that received 300 r alone. The birth weight differences in the 700-r, 17.5-day series (Table 2) are those between the controls and both the irradiated groups. In terms of 1-month survival, however, it seems obvious that the survival of any mice in the cysteinamine-treated group provides evidence for the drug's protective effect, for none of the 66 controls survived.

As can be seen from the data on survival and weight to 1 month after delivery, cysteinamine provides some protection against x-irradiation not only for the adult mouse, but also for the fetal mouse. This protection is expressed as a weight

Table 1. Data for mice irradiated with 300-r x-rays at 14.5 days of gestation. The percentage survival to age 1 month is based on the number alive at birth.

Treatment	Av. litter	Natal mortality (%)	Av. birth wt. (g)	Survival to 1 mo (%)	Av. wt. at 1 mo (g)
Cysteinamine alone (14 litters, 136 mice)	9.7	2.2	1.70	76.0	11.74
X-rays alone (6 litters, 50 mice)	8.3	14.0	1.18	40.0	6.83
Cysteinamine and x-rays (6 litters, 55 mice)	9.1	14.5	1.37	54.5	11.44

Table 2. Data for mice irradiated with 700-r x-rays at 17. 5 days of gestation. The percentage survival to age 1 month is based on the number alive at birth.

Treatment	Av. litter	Natal mortality (%)	Av. birth wt. (g)	Survival to 1 mo (%)	Av. wt. at 1 mo (g)
Cysteinamine alone (14 litters, 136 mice)	9.7	2.2	1.70	76.0	11.47
X-rays alone (13 litters, 66 mice)	5.1	3.0	1.25	0	
Cysteinamine and x-rays (12 litters, 92 mice)	7.7	8.7	1.30	78.6*	8.60

* Data for three litters.