parison of the eggshell curve in Fig. 1A and the curve for a leaf of the cottonwood, *Populus deltoides* (*17*) (Fig. 1B), shows the similarity. Although the near-IR reflectance of the leaf is less than 40 percent, the transmittance is about 50 percent so that the near-IR absorptance (absorptance is equal to 1 minus reflectance minus transmittance) of the leaf and eggshell are comparable (~10 percent). Some insects and certain hylid and centrolenid tree frogs (*1*, *18*) have spectral reflectance matching that of green vegetation in the near-IR but presumably lack the transmittance. The enhanced near-IR reflectance reported for the fur of the red kangaroo, *Megaleia rufa* (*19*), involves an extended, gradual transition. The pigmented avian egg thus appears to be unique among animal surfaces in its combination of cryptic visual coloration and thermoregulatory near-IR reflectance.

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References and Notes

1. H. B. Cott, *Adaptive Coloration in Animals* (Methuen, London, 1940), pp. 1–139.
2. L. von Haartman, *Evolution* **11**, 339 (1957).
3. W. A. Montevecchi, *Behaviour* **58**, 26 (1976).
4. R. E. Moreng and C. S. Shaffner, *Poult. Sci.* **30**, 255 (1951); A. F. Bennett and W. R. Dawson, in preparation.
5. R. H. Drent, in *The Herring Gull and Its Egg*, G. P. Baerends and R. H. Drent, Eds. (Brill, Leiden, 1970), pp. 104–109.
6. T. R. Howell and G. A. Bartholomew, *Ibis* **104**, 98 (1962); G. A. Bartholomew, *Condor* **68**, 523 (1966); T. R. Howell, B. Araya, W. R. Millie, *Univ. Calif. Berkeley Publ. Zool.* **104**, 1 (1974).
7. For reflectance measurements, we used a Beckman DK-2A spectrophotometer with an integrating sphere attachment and a $BaSO_4$ paint reference corrected to absolute reflectance against a pressed $BaSO_4$ powder standard as described by F. Grum and G. W. Luckey [*Appl. Opt.* **7**, 2289 (1968)].
8. Courtesy of M. P. Thekaekara and the National Space Science Data Center, Goddard Space Flight Center, Greenbelt, Md.
9. The Heermann's gull material was obtained during a study of a breeding colony located on Isla Rasa, Baja California Norte, Mexico. Studies in the colony were conducted from 8 to 30 April 1976.
10. W. P. Porter, *Ecol. Monogr.* **37**, 273 (1967).
11. G. Y. Kennedy and H. G. Vevers, *Comp. Biochem. Physiol. B* **44**, 11 (1973).
12. All eggs were from museum collections, and most of the blue eggs had faded noticeably. Only unfaded examples were measured. Little fading of brown pigments was noted in a comparison of recently collected and 90-year-old eggs of herring gulls, *Larus argentatus*.
13. G. Y. Kennedy and H. G. Vevers, *Comp. Biochem. Physiol. B* **55**, 117 (1976).
14. We calculated solar absorptances by using a finite approximation to equation 3 of W. P. Porter and D. M. Gates [*Ecol. Monogr.* **39**, 227 (1969)]. Intact eggs would probably show stronger absorption in the liquid water bands than the dry museum eggshells measured. However, most solar radiation in these bands is absorbed by atmospheric water vapor before reaching the ground, so that the effective solar absorptance is little affected. The spectral absorptance of mantle feathers was measured on the center of the back, and the spectral absorptance of contour feathers was measured on the center of the breast.
15. See G. S. Bakken and D. M. Gates [in *Perspectives of Biophysical Ecology*, D. M. Gates and R. B. Schmerl, Eds. (Springer, New York, 1975), pp. 255–290] for a discussion of the theory, measurement, and history of T_e.
16. These copper-lined eggs were prepared from eggs freshly obtained in the Heermann's gull colony. The egg contents were washed out with water through a small window in the shell. The interior of the shell was then coated with an electrically conductive paint. Copper was then electroformed over this painted surface according to the technique described in (*15*).
17. D. M. Gates, *Am. Sci.* **51**, 327 (1963);_____, H. J. Keegan, J. C. Schleter, V. R. Weidner, *Appl. Opt.* **4**, 11 (1965).
18. P. A. Schwalm, P. H. Starrett, R. W. McDiarmid, *Science* **196**, 1225 (1977).
19. T. J. Dawson and G. D. Brown, *Comp. Biochem. Physiol.* **37**, 23 (1970).
20. We thank R. B. Payne, R. W. Mumford, and H. R. Rahn for arranging the loan of material from the collections of the University of Michigan, Purdue University, and the State University of New York at Buffalo, respectively. We also thank G. W. Issacs for arranging for us to use the Purdue University Department of Agricultural Engineering Beckman DK-2A spectrophotometer. This work was supported by an Indiana State University faculty research grant to G.S.B. and NSF grant DEB-76-7817 and BMS-75-3300 to W.R.D. Our trip to Isla Rasa was made on R.V. *Dolphin* (Scripps Institution of Oceanography, University of California, San Diego). We thank B. Villa-Ramirez for valuable assistance and the government of Mexico (Office of the Secretary of External Relations) for permission to work on Isla Rasa and collect eggs (permit 502113, 20 March 1976).

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Myotonic Muscular Dystrophy: Altered Calcium Transport in Erythrocytes

Abstract. *Erythrocytes from patients with myotonic muscular dystrophy accumulate calcium at a significantly higher rate than normal controls do. This increased rate of net accumulation appears related to an enhanced permeability of the membrane to calcium, rather than to an impairment in its active outward transport.*

Myotonic muscular dystrophy (MyMD) is an inherited disease of man characterized by a variable clinical presentation which may include myotonia, weakness and atrophy of skeletal muscle, dysphagia and constipation, cardiac arrhythmias, testicular atrophy, hyperinsulinemia in response to a glucose load, cataracts, frontal balding, and rapid turnover of immunoglobulins (*1*). Although the specific inborn error of metabolism is not known, recent data suggest that the cellular plasma membranes in many organ systems are disturbed.

Electrophysiological studies of skeletal muscle have ascribed the myotonia of MyMD to alterations of muscle surface membrane (*2*). These data have been supported by the morphological demonstration through freeze fracture analysis of an increased number of intramembranous particles (*3*). Similar studies of other myotonic syndromes in man, such as myotonia congenita and drug-induced myotonia, have also localized the abnormality to surface membranes (*4*, *5*). Although the myotonia of these latter conditions can be best explained by a decrease in chloride conductance, the myotonia of MyMD is not accompanied by any change in membrane resistance or conductance (*2*), and therefore an alternate mechanism must be investigated.

Previous studies have demonstrated that red blood cells of patients with MyMD exhibit definite membrane abnormalities, including increased membrane fluidity (*6*, *7*), impaired protein phosphorylation (*8*), and abnormal shape when viewed through the scanning electron microscope (*9*). More recently, we have demonstrated an alteration in the calcium promoted potassium efflux in MyMD red blood cells exposed to iodoacetic acid and adenosine (*10*). Although these experiments only examined potassium movements, they did suggest a potential alteration in transmembrane calcium movements.

The availability of improved techniques for measuring calcium transport made possible our studies on the red blood cells of patients with MyMD. In comparison with normal controls, the red blood cells from six out of seven patients with myotonic dystrophy were observed to have a significantly increased rate of calcium accumulation and an increased rate of calcium efflux against an electrochemical gradient.

All studies were performed with heparinized blood from fasted subjects. The red cells were separated and washed with isotonic tetramethylammonium chloride (0.17*M*) adjusted with 5 m*M* tris buffer, *p*H 7.4, 20°C. Cells were washed three times with this solution for accumulation studies, six times for the measurement of calcium accumulation after incubation, and six times without incubation for the starting calcium values. The patient population consisted of seven males with moderately severe myotonic muscular dystrophy. Control samples were obtained from healthy males of similar ages. None were anemic. Both patient and control samples were ob-

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Table 1. Calcium accumulation in erythrocytes from myotonic muscular dystrophy and control subjects. The incubation medium contained 40 m*M* calcium chloride, 10 m*M* sodium chloride, 1 m*M* potassium chloride, 10 m*M* glucose, 20 m*M* Hepes-tris buffer adjusted to *p*H 7.8 at 20°C, and sufficient tetramethylammonium chloride to provide a final osmolality of 300 milliosmoles per kilogram of solution. Calcium measurements were done with an atomic absorption spectrophotometer equipped with a heated graphite atomizer (*12*). Rates of accumulation were obtained by duplicate measurements of the calcium content of erythrocytes at 15-, 45-, and 75-minute incubation and are expressed as the average rate (micromoles per liter) of accumulation over this 60-minute period. Group results are expressed with their standard errors. The average correlation coefficient for both groups was 0.97 (*13*).

Control		MyMD	
Subject	Rate	Subject	Rate
S.A.	32.6	J.E.	57.0
W.C.	39.3	J.S.	62.0
J.B.	50.2	J.L., Sr.	58.6
S.E.	28.3	J.L., Jr.	55.1
G.P.	26.4	R.W.	65.0
H.G.	39.4	T.H.	65.0
S.G.	35.2	P.P.	43.7
	$\bar{X}$ = 35.9 ±3.0		$\bar{X}$ = 58.1 ±2.8

Table 2. Calcium efflux from myotonic and normal subjects. In each study the erythrocytes of both groups were loaded to approximately the same concentration of calcium by incubation for 1 hour at 20°C in hypertonic calcium solutions (*14*). The incubation media of the efflux studies contained either less than 5 μM calcium (study 1) or 250 μM calcium (studies 2 and 3), and 10 m*M* sodium chloride, 1 m*M* potassium chloride, 10 m*M* glucose, 20 m*M* Hepes-tris buffer adjusted to *p*H 7.8 at 37°C, and sufficient tetramethylammonium chloride to provide a final osmolality of 300 milliosmoles per kilogram of solution. The reaction mixtures were incubated at 37°C. The rate of efflux was obtained by duplicate measurements of the calcium content of erythrocytes 15, 45, and 75 minutes incubation and are expressed as the average rate (micromoles per liter) of efflux over this 60-minute period. The effluxes were linear over this period. Group results are expressed with their standard errors. Both groups had average correlation coefficients of 0.97.

Study	Control		MyMD	
	Subject	Rate	Subject	Rate
1	G.H.	20.5	J.E.	32.9
2	M.T.	17.0	R.W.	45.3
3	G.P.	25.0	J.S.	29.4
			P.P.	24.6
		$\bar{X}$ = 20.8 ± 2.3		$\bar{X}$ = 33.1 ± 4.4

tained at the same time and tested in identical fashion. The mixtures were incubated with moderate shaking at a cell to medium ratio between 1 to 10 and 1 to 20. At these ratios, the sodium and potassium concentrations in the medium remain constant during a 2-hour incubation. All accumulation studies were performed at 20°C (Tables 1 and 2).

Erythrocytes from both the controls and the patients displayed a linear rate of calcium accumulation during the 60-minute incubation period (Table 1). The rate of accumulation for the group of patients was significantly higher ($P < .001$, unpaired *t*-test) than the average normal control rate.

To determine whether this enhanced accumulation was due to an altered starting value, we examined in separate studies the calcium content of erythrocytes from five myotonic and seven normal subjects. The mean calcium content of the myotonic cells was 16.6 ± 0.8 μmole/liter, which was not significantly different from the control value of 14.5 ± 0.8 μmole/liter.

We then examined the question of whether the increased accumulation was related to an augmented influx or an impaired efflux of calcium in myotonic cells by testing the efflux rates of calcium-loaded erythrocytes from control and myotonic patients (Table 2). In the first efflux study, the rate of loss was determined in the absence of added calcium in the incubating solution; in two additional efflux studies, the external calcium concentration was raised to 250 μM. This concentration is higher than that of calcium-loaded erythrocytes, so that, even if all the calcium within the erythrocytes were ionized, the efflux of the cation would occur against an electrochemical gradient. With both groups the efflux of calcium was linear over the period studied. The average rate of calcium efflux from the myotonic group was significantly higher ($P < .05$, unpaired *t*-test) than the average control rate. One myotonic subject (P.P.) displayed an efflux rate within the normal range, as well as the normal influx rate noted above. These studies established that the increased accumulation observed during calcium loading conditions in vitro was related to an increased permeability of the membrane to calcium rather than to an impairment of active efflux.

The effect of quinine on calcium accumulation was studied on normal and MyMD red blood cells. Quinine was selected because it produced clinical improvement in human myotonia and because it is known to block calcium promoted potassium efflux (*11*). In our studies, quinine decreased calcium accumulation in both normal controls and in patients. In the presence of 0.1 m*M* quinine, the average rates of accumulation were reduced to 13.5 ± 2.1 μmole/liter per hour for three control subjects and to 16.1 ± 3.5 μmole/liter per hour in three myotonic patients. The similarity of these rates suggested that the quinine sensitive influx rate may be responsible for the enhanced accumulation of calcium noted in red blood cells from myotonic patients.

These observations provide evidence for a membrane disturbance in the movement of calcium in patients with myotonic dystrophy. Whether the increased accumulation and efflux of calcium from red blood cells are secondary manifestations of such a disturbance or essential features of the myotonic process in muscle is unknown. Furthermore, it is unclear whether abnormal calcium movements can give rise to the cataracts, cardiac arrhythmias, endocrine abnormality, and repetitive depolarization of the muscle membrane noted in myotonic dystrophy. Nevertheless, the fundamental role of calcium in many intracellular processes, including its critical role in muscle function, suggests that an impairment in its precise regulation could lead to such widespread effects.

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References and Notes

1. J. E. Caughey and N. C. Myrianthopoulus, *Dystrophia Myotonica and Related Disorders* (Thomas, Springfield, Ill., 1963), p. 282.
2. R. J. Lipicky and S. H. Bryant, in *New Developments in Electromyography and Clinical Neurophysiology*, J. E. Desmedt, Ed. (Karger, Basel, 1973).
3. D. L. Schotland and E. Bonnila, *Neurology* **27**, 379 (1976).
4. S. H. Bryant, in *Membranes and Disease*, L. Bolis, J. F. Hoffman, A. Leaf, Eds. (Raven, New York, 1976), p. 197.
5. R. Rudel, in *ibid.*, p. 207.
6. D. A. Butterfield, A. D. Roses, M. L. Cooper, S. H. Appel, D. B. Chesnut, *Biochemistry* **13**, 5078 (1974).
7. D. A. Butterfield, A. D. Roses, D. B. Chesnut, S. H. Appel, *Trans. Am. Soc. Neurochem.* **6**, 163 (1975).
8. A. D. Roses and S. H. Appel, *J. Membr. Biol.* **20**, 51 (1975).
9. S. E. Miller, A. D. Roses, S. H. Appel, *Arch. Neurol.* **33**, 172 (1976).
10. S. H. Appel and A. D. Roses, in *Membranes and Disease*, L. Bolis, J. F. Hoffman, A. Leaf, Eds. (Raven, New York, 1976), p. 183.
11. M. Armando-Hardy, J. C. Ellory, H. G. Ferrira, S. Fleminger, V. L. Lew, *J. Physiol. (London)* **250**, 32P (1975).
12. J. C. Parker, H. J. Gitelman, P. S. Glosson, D. L. Leonard, *J. Gen. Physiol.* **65**, 84 (1975).

13. We have observed that the potassium content of normal erythrocytes shows a less than 6 percent decrease during such incubations. When glucose is present, there is a loss of approximately 20 percent of the starting amount of adenosine triphosphate (ATP). This loss is not altered by the presence of calcium in the incubation solution. In the absence of glucose, there is no alteration in ATP content or the degree of calcium accumulation (G. A. Plishker and H. J. Gitelman, in preparation).

14. G. Plishker and H. J. Gitelman, *J. Gen. Physiol.* **68**, 29 (1976).

15. We thank R. Cole and W. J. Clingenpeel for technical assistance. Supported by grants from the Muscular Dystrophy Association and the National Institutes of Health.

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Aflatoxicol: Major Aflatoxin B_1 Metabolite in Rat Plasma

Abstract. *Aflatoxicol, a carcinogenic metabolite of the foodborne carcinogen aflatoxin B_1 previously known only as a bioreduction product in vitro, was identified as the major aflatoxin metabolite in the plasma of Sprague-Dawley rats, a susceptible species, that had been dosed orally or intravenously with aflatoxin B_1 labeled with carbon-14. Aflatoxicol, however, was not detected in the plasma of similarly dosed mice and monkeys, which are both resistant to aflatoxin B_1–induced carcinogenesis. The formation of aflatoxicol both in vitro and in vivo may be an indicator of species sensitivity to aflatoxin-induced carcinogenesis and may be useful in the prediction of human susceptibility.*

Aflatoxin B_1 (AFB_1), a mycotoxin produced by certain strains of *Aspergillus*, has been recognized as a significant food contaminant because of its widespread occurrence in such foodstuffs as peanuts and corn (*1*) and its potent hepatocarcinogenicity in rats and other test animals (*2*). The concern over AFB_1 is substantiated by epidemiologic studies which show a correlation between the current incidence of primary liver cancer in certain human populations and the ingestion of AFB_1-contaminated diets (*3*).

Recently, studies of metabolism and mode of action have provided evidence that AFB_1 requires metabolic activation to elicit its carcinogenic effects (*4*), that the ultimate carcinogenic form may be the 8,9-epoxide of AFB_1 (*5, 6*), and that the marked differences in species susceptibility to the carcinogenic effects of AFB_1 can be correlated with species differences in the metabolism of AFB_1 (*7, 8*).

Aflatoxin B_1 can be oxidized in vitro by liver microsomal oxygenases to AFM_1, AFQ_1, AFP_1, and AFB_{2a} (*9*); AFB_1 and AFQ_1 can also be reduced by cytoplasmic reductase to aflatoxicol (AFL) (*10, 11*) (Fig. 1) and $AFLH_1$ (*12*), respectively. Since these metabolites have distinctly different mutagenic activities (*13*), it is reasonable to assume that the carcinogenicity of AFB_1 is influenced by its ultimate partitioning among these competing metabolic pathways if they are operative in vivo. On the basis of metabolism data obtained in vitro, we have found that the susceptibility of a species to the carcinogenic effect of AFB_1 is directly correlated with hepatic transformation of AFB_1 to AFL but inversely correlated with conversion of AFB_1 to AFQ_1 or to aqueous metabolites (*14*). This observed correlation between AFL formation and species susceptibility to aflatoxin-induced carcinogenesis may provide a means for estimating human susceptibility.

Despite these interesting findings, to our knowledge AFL has not previously been isolated in vivo to support a relationship between the biotransformation in vitro and the metabolism and disposition in vivo of AFB_1. So far, of the six metabolites mentioned, only AFM_1 has been consistently isolated from the excreta and tissues of exposed animals (*15*) and humans (*16*). However, in the study reported here, we found that AFL was the major AFB_1 metabolite in the plasma of rats dosed with AFB_1 either intravenously or orally. The identification of AFL as a metabolite of AFB_1 in vivo not only confirms the significance of metabolism studies in vitro but also strengthens the possibility that the AFL-forming activity can be used as a metabolic parameter to estimate species susceptibility to aflatoxin-induced carcinogenesis.

Experiments were conducted with Sprague-Dawley rats weighing 260 to 280 g. The rats were anesthetized with diethyl ether and secured dorsally to a heated surgical platform, and both jugular veins were revealed by blunt dissection for catheterization with a 27-gauge needle cannula. To study the toxicokinetics of AFB_1, 1.0 mg/kg of ring-labeled [^{14}C]AFB_1 (0.6 Ci/mole), dissolved in 0.3 ml of a mixture of physiological saline solution and dimethyl sulfoxide (3:1), was administered intravenously as a rapid infusion into the left jugular vein. Blood samples were then collected every 5 to 10 minutes for 50 minutes from the opposing catheterized jugular vein. In studies where AFB_1 was administered orally by intubation, the dose was tripled and blood samples were taken for 100 minutes at wider intervals.

Approximately 95 percent of the total blood radioactivity was found in the plasma, and the total plasma radioactivity was partitioned into the protein, chloroform-extractable, and residual aqueous fractions. Time-concentration profiles of various radioactive species in the plasma of rats administered [^{14}C]AFB_1 are shown in Fig. 2. In the rats dosed intravenously, the major identifiable constituents of the chloroform-extractable fraction were residual AFB_1, AFM_1, and a metabolite subsequently identified as AFL. Aflatoxin M_1, which is invariably the major identifiable urinary metabolite, was present only in trace amounts, accounting for less than 0.2 percent of the total plasma radioactivity. Aflatoxicol was found to be the principal metabolite, representing 15 to 25 percent of the total plasma radioactivity and consistently accounting for more than 25 percent of the chloroform-extractable radioactivity.

The major plasma metabolite was identified as AFL by thin-layer chromatography (TLC) and ultraviolet and mass spectroscopic analyses, using AFL standards prepared by biotransformation in vitro (*10, 11*) and by chemical reduction of AFB_1 (*17*). Analytical evidence for the bioreduction of AFB_1 to its cyclopentanol structure, AFL, is summarized in Table 1. The identification of AFL was further confirmed by its relatively potent mutagenic activity to *Salmonella typhimurium* strain TA98 upon metabolic activation (*13*).

It was observed that oral administration of [^{14}C]AFB_1 resulted in a much lower total plasma radioactivity, which peaked at 1 hour (Fig. 2). The concentrations of AFL and AFM_1 relative to that

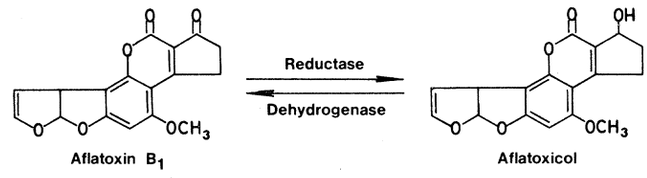

Fig. 1. Interconversion of aflatoxin B_1 and aflatoxicol. Aflatoxicol is formed by reduction of the cyclopentanone function of aflatoxin B_1.

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