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ORGANIC DYES AND STRYCHNINE-MOLYBDATE REAGENT AS AIDS IN ISOLATING PHOSPHATE MINERAL GRAINS

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ABSTRACT

Recognition of individual phosphate grains is enhanced by a procedure that isolates the grain but does not destroy it. Numerous organic dyes were tested and evaluated as to their phosphate-crystal-staining capacity. No one dye was found to stain all phosphates.

Seventy phosphate minerals were used to evaluate a strychnine-molybdate qualitative test. All phosphate grains tested formed a blue complex with the reagent. A procedure is presented for the application of the strychnine-molybdate reagent.

INTRODUCTION

One of the first problems encountered in attempting to determine the phosphate mineralogy of a soil or sediment is that the phosphate minerals are normally a minor component. The phosphorus content of a soil, for example, seldom exceeds a few tenths percent. This makes a direct mineralogical analysis of the phosphate phase almost impossible. Concentration of the phosphate phase is not normally possible because the phosphate minerals as a group have no suitable distinctive physical properties. Orthophosphates do undergo distinctive chemical reactions, however, with possibilities for selective reactions with suitable reagents or dyes that might permit the concentration of phosphate grains by selection. The objective of this investigation was to develop such a procedure.

Some fundamental limitations should be noted at the outset. First, the use of a stain or complexing reagent requires particles large enough to permit color evaluation in the microscope field. Second, identification of stained grains frequently requires that they be removed from the field by some type of probing technique. In both instances work is usually limited to grains exceeding 10 μ diameter. Third, common techniques such as petrographic analysis and X-ray diffraction are limited to crystal-line particles, and identifications are limited to those mineral species for which suitable diagnostic data are procurable.

Staining tests for minerals such as feldspars and layer silicates are available (see for example Jackson, 1956; Krumbein and Pettijohn, 1938), but, chemical tests or staining procedures for phosphate minerals are rare. The few discovered were not designed to indicate which particular grains are phosphates. Rather, they were designed to indicate whether or not phosphate was present by dissolving a portion of the sample and forming colored complexes (see Feigl, 1954, for a laboratory procedure and Oaks, 1938, for a field procedure). In contrast to mineral stains, there are quite a few organic dyes that are used to indicate sites of phosphatase activity in biological sections (for example, Picard and Vitry, 1956). Organic dyes seem not to have been evaluated from the standpoint of phosphate mineral stains, so, it is possible that some worthwhile reagents have been overlooked. Since organic dyes may color the surface of a phosphate grain (rather than dissolve a portion of it and form a colored complex elsewhere), they are worth investigation.

MATERIALS

Reagents. All chemicals are reagent grade unless otherwise noted.

2% gelatin. Dissolve 0.5 g USP gelatin powder in 25 ml warm water (80°C). This reagent is dependable for only two or three days due to the growth of microorganisms.

1% SnCl₂·2H₂O. Dissolve 0.25 g SnCl₂·2H₂O in 1 ml of 12 N HCl. Mix with 25 ml water and store in brown dropper bottle. This reagent should be freshly made.

Strychnine-molybdate solution.¹ To 87 ml of H₂O in a 250 ml beaker, add 13 ml of 16 N HNO₃ and mix. Then dissolve 2.0 g MoO₃. Next add 0.6 g strychnine nitrate (K & K laboratories, Inc. 177–10 93rd Ave., Jamaica 33, New York) and heat gently until no further dissolution is observed. Filter if necessary. The reagent was found to last over 8 months in a polyethylene bottle.

Apparatus

Filter disc. A 1-inch diameter Nylon filter disc and ring and Geon filter stopper from Control Molding Corp. 84 Granite Ave. Staten Island 3, New York, worked well. Filters cut from a hard paper such as Whatman No. 52 entrap few grains in the mesh.

Wire loop. A satisfactory grain manipulator was constructed from 1 mil tungsten wire (from S. R. Blumberg, General Electric Co., Cleveland, Ohio). About one-half inch of the wire is doubled over and the free ends cemented to a suitable holder. To pick up a grain, dip the loop in glycerol, wipe the excess off on a clean surface, and touch the end of the loop to the grain. The grain will part from the loop when inserted into a drop of water.

EXPERIMENTAL

Organic dyes. A perusal of the literature indicated nile blue (Drews, 1956) to be a promising stain for several substances containing organic phosphorus. As an initial test on minerals, it was found that portions of the dufrenite, monazite, triphylite, wavellite, and pyromorphite samples were stained a shade of blue by the application of a 0.07% solution of nile blue A in alcohol. Quartz and hematite remained unstained. These results were sufficiently encouraging to warrant a more extensive inves-

¹ Personal communication from J. R. Lehr, TVA, Wilson Dam, Alabama.

tigation of the mineral staining characteristics of nile blue and other organic dyes.

Approximately 1 ml of a 0.20% solution of each dye in alcohol was applied to about 25 grains from each of three mineral mixtures in glass vials. Gibbsite grains were treated separately. The first mixture contained the phosphates apatite, dufrenite, variscite, and wavellite; the second contained the silicates quartz, feldspar and vermiculite; the third contained the iron oxides hematite and limonite. The dye solution was allowed to contact the mineral samples for 1.0 minute. The excess dye was removed by diluting with water and removing the supernatant liquid with a filter stick. The dilution was continued until the supernatant liquid appeared colorless. The mineral grains on the bottom of the glass vials were examined for staining effects with a microscope. The only member of the silicate mixture that stained was vermiculite, with the dyes absorbed at the flake edges. None of the dyes stained limonite, hematite, or gibbsite. Of the phosphates, apatite was not stained, but about half of the dyes stained the dufrenite, variscite, and wavellite samples to varving degrees (Table 1).

Practically all the variscite and wavellite grains were stained by only three dyes each, although some material present in the wavellite samples was stained by almost every dye. The dufrenite sample was stained more often than the other two phosphates and appeared to contain a minor component that stained darker than the major component. Also, the variscite sample had a small amount of material that stained a completely different color from the greater part of the sample.

It seems evident from these results that no organic dye is likely to be found that will serve as an indicator of all phosphate minerals.¹ However,

¹ The dyes that did not stain an	y component of the	phosphate samples tested were:
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acid fuchsin	erythrosin, yellowish	orseillin BB
amaranth	ethyl eosin	patent blue V
anthracene blue SWX	flavianic acid	phenolphthalein
atabrine	fluorescein	phloxine B
auramine O	gallocyanin	primulin
azo blue	gallamin blue	purpurin
azolitmin	hematein	quinalizarin
benzopurpurin 4B	indigo-carmine	quinoline
bindschedler's green	indigo tetrasulfonate	resazurin
bismark brown Y	isamine blue	sudan black B
brazillin	lacmoid	sudan III
brilliant purpurin R	martius yellow	sudan IV
brom phenol blue	methyl orange	tetrazolium blue
brom thymol blue	muci-carmine	tetrazolium red
carminic acid	narcein	tetrazolium violet
cochineal	neotetrazolium	thiazine red R
congo red	nigrosin W	toluidine blue O
eosin B	oil blue NA	trypan red
eosin Y	oil red O	vital red
erythrosin B	orange G	

	Q	Λ	M	Dye	Q	Λ	Μ	Dye	D	Λ	M
alizarin	0	+	0	hofmann's violet	+	*	*	nroain	c	c	*
alizarin red S	0	+	*	iodine green		0	*	when a free to	> -	⊃ *	0.9
amethyst violet	C	0	*	1 D			4	pueno-santanin	+	ł	ŧ
		> +	5 A	Janus green b	┝	0	6 3	pinacyanol	+	*	¥
Dasic Iuchsin	0	÷	ĸ	light green FS yellowish	0	0	*	pyronin B	0	0	*
brilliant cresyl blue	ł	0	*	magdala red	0	0	*	pvronin-methyl green	0	*	*
brilliant green	┿	0	*	malachite green	+	0	×	rhodamine 6G	+	0	*
brom cresol purple	0	0	+	methyl blue	+	*	*	safranin 0	- 0	0	*
celestin blue B	+	*	*	methyl green	+	0	*	spirit blue	+	0	*
chrysoidin R	0	0	*	methyl red	0	0	*	thioflavine S	0) *	*
chrysoidin Y	0	*	*	- methylene blue	+	*	*	thioflavine T	0	0	*
cresyl violet	0	+	*	methylene green	+	0	*	thionin	0	*	*
crystal violet	0	0	*	methylene violet	+	*	*	toluvlene blue	0	0	*
dianil blue 2R	0	*	*	methylene violet WS	0	0	*	victoria blue B	· +	*	*
erythrosin B	0	0	×	neutral red	0	0	×	victoria blue 4R	- 0	*	*
evans blue	+	*	+	new blue R	0	0	*	victoria blue R		0	*
fast green FCF	0	0	+	nigrosin (alc. sol.)	+	*	*	wright stain	> -	*	*
giemsa stain	0	0	*	nile blue A	0	*	*				
	0 = 1	None s	tained.					D - Archanita			
)= *	Only a	few grai	ns or parts of grains stained				V = variscite.			
	1 = +	ractic	ally all c	f the grains stained.				W=wavellite.			

TABLE 1. DYES THAT STAINED PHOSPHATE MINERALS

266

J. A. KITTRICK AND E. W. HOPE

ISOLATING PHOSPHATE GRAINS

where one is dealing with material that is predominantly phosphate, it may be possible to select a dye, or a series of dyes, that would permit a distinction between the various components in the phosphate sample. An example of this occurred when a sample of synthetic variscite, used in a series of solubility studies, was treated with iodine green. The portion of the variscite sample that stained blue-green gave a X-ray diffraction pattern very similar to the one given by Claringbull on card 8-157 of the ASTM X-Ray Powder Data File. However, when some of the unstained sample was separated and X-rayed, the 6.31 and 4.54 Å lines (present in the stained sample and on the ASTM card) were absent. These lines are not indexed on the ASTM card and are probably due to an impurity.

Colored phosphate complexes. It seems likely that a test for phosphate minerals as a group will have to be based upon a distinctive phosphate reaction. The two best known phosphate complexes and the bases for most phosphate chemical analyses are molybdenum blue and yellow phosphovanadomolybdate. Preliminary experiments with both tests indicated that the molybdenum blue color is more easily recognized under the microscope, so, further efforts were concentrated on it.

The spot test described by Feigl (1954) for the detection of phosphate in minerals and rocks was used as a starting point. The test is designed primarily to indicate the presence of apatite in a rock or mineral sample. The sample (p. 438) "... is placed on filter paper, moistened with a drop of ammonium molybdate solution and held over a flame for a short time. A drop of benzidine solution is added and the moist fleck held over ammonia." A blue color forms in the filter paper. Unfortunately this test did not seem to give a usable blue color with phosphates other than apatite.

A more sensitive test was found which uses a strychnine-molybdate reagent with $SnCl_2$ as the reductant; it was also found to be less influenced by experimental conditions. First rubber cement and then an acrylic plastic (spray) was used to hold the mineral grains in place on the filter paper. Later gelatin was found to hold the crystals well and at the same time to limit diffusion of the blue color. This made it much easier to tell which grain was the source of the blue color.

Relatively insoluble phosphates dissolve sufficiently in the acid $(2 N \text{ HNO}_3)$ strychnine-molybdate reagent to permit the formation of a visible blue color without undue dissolution of the mineral grain. However, it soon became apparent that the more soluble phosphate minerals could dissolve completely. Some regulation of the contact time between mineral grain and fresh reagent was necessary. Mineral dissolution was at

first controlled by brushing on the strychnine-molybdate reagent. Later, a fine spray was found to be an improvement. Finally, the sample and filter paper were placed on a filter disc, the reagent added with a dropper, and contact time regulated by varying the amount of suction.

Phosphate mineral test procedure. The Geon stopper with the Nylon filter disc and filter paper is inserted into a small filtering flask which is connected to a vacuum source. Place this apparatus under a suitable microscope (a $30 \times$ stereoscopic wide field binocular is very convenient): focus on the filter paper surface and subsequent operations. Turn the suction on and wet the filter paper with three drops of 2% gelatin solution and then by an equal amount of SnCl₂ solution. To the filter paper, transfer 20-50 sample grains that have been size-separated to a narrow particlesize range using fine sieves and a minimum of chemical pretreatment. Small particles, can be conveniently transferred by inserting a toothpick firmly into the sample vial and then dislodging the adhering grains as evenly as possible by gently tapping the toothpick. Wet the filter paper and grains with two or three drops of strychnine-molybdate solution. In a few seconds the filter paper will turn blue near the very soluble phosphate grains. These grains should be removed immediately upon detection of color and placed in a drop of water (spot plate) to prevent excessive dissolution. Small grains can be handled with a fine wire loop moistened with glycerol. After removing the very soluble grains an additional one or two applications of the strychnine-molybdate and SnCl₂ reagents can be given and the reacting grains removed. All phosphate grains are then washed in a drop of water or acetone to remove reagents.

Test of the strychnine-molybdate reagents on standard minerals. If a phosphate mineral test is to have wide application, it is necessary that it react with essentially all phosphate minerals. Accordingly, a group of phosphate minerals representing a wide range in solubilities and associated cations was tested with the strychnine-molybdate reagent (Table 2). All gave a positive test. It is also necessary that nonphosphate minerals common to soils and sediments do not give a positive test, which was the case with the minerals listed in Table 3. While testing the reagents on nonphosphate minerals, a few samples gave positive tests, apparently due to phosphate contamination. In the case of magnetite (New York), the magnetite (which did not give a positive test) could easily be separated from the phosphate impurity under the binocular microscope. In the case of augite (Quebec), calcite (Fischer C. P. CaCO₃), and microcline (S. Dakota), samples of similar minerals from Cargille did not give a positive test.

ISOLATING PHOSPHATE GRAINS

Mineral ¹	Formula ²	Locality Sup	plier
alluaudite	(Na, Fe ³⁺ , Mn ²⁺)(PO ₄)	South Dakota	3
amblygonite	(Li, Na)Al(PO ₄)(F, OH)	South Dakota	1
anapaite (messelite)	$Ca_2Fe(PO_4)_2 \cdot 4H_2O$	New Hampshire	4
arrojadite	Na ₂ (Fe ²⁺ , Mn ²⁺) ₅ (PO ₄) ₄	South Dakota	3
augelite	$Al_2(PO_4)(OH)_3$	South Dakota	3
barbosalite	$Fe^{2+}Fe^{3+}(PO_4)_2(OH)_2$	South Dakota	3
beraunite	Fe ²⁺ Fe ₄ ³⁺ (PO ₄) ₃ (OH) ₅ ·3H ₂ O	Arkansas	6
brazilianite	$NaAl_3(PO_4)_2(OH)_4$	Brazil	10
brushite	$CaHPO_4 \cdot 2H_2O$	synthetic	9
chalcosiderite	$CuFe_6(PO_4)_4(OH)_8 \cdot 4H_2O$	England	4
childrenite	(Fe ²⁺ , Mn ²⁺)Al(PO ₄)(OH) ₂ ·H ₂ O	England	4
collinsite	Ca ₂ (Mg, Fe)(PO ₄) ₂ ·2H ₂ O	British Columbia	12
corkite	$PbFe_3(PO_4)(SO_4)(OH)_6$	Mexico	5
crandallite	$CaAl_3(PO_4)_2(OH)_5 \cdot H_2O$	Germany	12
diadochite	$Fe_2(PO_4)(SO_4)(OH) \cdot 5H_2O$	California	4
dufrenite	$Fe^{2+}Fe_{4}^{3+}(PO_{4})_{3}(OH)_{5} \cdot 2H_{2}O$	Maryland	1
eosphorite	(Mn ²⁺ , Fe ²⁺)Al(PO ₄)(OH) ₂ ·H ₂ O	New Hampshire	3
evansite	$Al_3(PO_4)(OH)_6 \cdot 6H_2O$	Spain	5
fairfieldite	Ca ₂ (Mn, Fe)(PO ₄) ₂ ·2H ₂ O	Connecticut	8
florencite	CeAl ₃ (PO ₄) ₂ (OH) ₆	S. W. Africa	4
fluorapatite	$Ca_5(PO_4)_3F$	Quebec	1
frondelite	(Mn ²⁺ , Fe ²⁺)Fe ³⁺ (PO ₄) ₃ (OH) ₅	Germany	4
graftonite	$(Fe, Mn, Ca)_3(PO_4)_2$	South Dakota	2
griphite	(Na, Al, Ca, Fe) ₃ Mn ₂ (PO ₄) _{2.5} (OH, F) ₂	South Dakota	3
hagendorfite	(Na, Ca)(Fe, Mn) ₂ (PO ₄) ₂	Germany	2
herderite	$CaBe(PO_4)(F, OH)$	New Hampshire	5
heterosite	(Fe ³⁺ , Mn ³⁺)(PO ₄)	South Dakota	3
hureaulite	$Mn_5H_2(PO_4)_4 \cdot 4H_2O$	South Dakota	3
isokite	CaMgPO ₄ F	North Rhodesia	4
lazulite	$(Mg, Fe^{2+})Al_2(PO_4)_2(OH)_2$	Georgia	3
libethenite	$Cu_2(PO_4)(OH)$	Nevada	4
lithiophilite	$Li(Mn^{2+}, Fe^{2+})(PO_4)$	South Dakota	3
ludlamite	(Fe ²⁺ , Mg, Mn) ₃ (PO ₄) ₂ ·4H ₂ O	Idaho	3
messelite	$Ca_2Fe(PO_4)_2 \cdot 2\frac{1}{2}H_2O$	Germany	2
meta-autunite	$Ca(UO_2)_2(PO_4)_2 \cdot 6\frac{1}{2} - 2\frac{1}{2}H_2O$	Washington	3
metastrengite	$FePO_4 \cdot 2H_2O$	California	6
monazite	(Ce, La, Y, Th)(PO ₄)	New Mexico	1
monazite	(Cd, La, Y, Th)(PO ₄)	Idaho	11
montebrasite	(Li, Na)Al(PO ₄)(OH, F)	South Dakota	3
morinite	$Na_2Ca_4Al_4(PO_4)_4(OH)_4F_6\cdot 3H_2O?$	South Dakota	3
parahopeite	$Zn_3(PO_4)_2 \cdot 4H_2O$	Rhodesia	2
paravauxite	$FeAl_2(PO_4)_2(OH)_2 \cdot 8H_2O$	Bolivia	3
parsonite	$Pb_2(UO_2)(PO_4)_2 \cdot 2H_2O$	France	6
phosphoferrite	$(Fe, Mn)_3(PO_4)_2 \cdot 3H_2O$	Germany	2
phosphophyllite	$Zn_2(Fe, Mn)(PO_4)_2 \cdot 4H_2O$	Bolivia	5

TABLE 2. PHOSPHATES THAT GAVE A POSITIVE TEST WITH THE STRYCHNINE-MOLYBDATE REAGENT

$Mineral^1$	Formula ²	Locality Sup	plier ³
pseudomalachite	$Cu_5(PO_4)_2(OH)_4 \cdot H_2O$	North Rhodesia	4
purpurite	$(Mn^{3+}, Fe^{3+})(PO_4)$	New Hampshire	4
pyromorphite	$Pb_{5}(PO_{4})_{3}Cl$	Idaho	1
reddingite	$(Mn, Fe)_3(PO_4)_2 \cdot 3H_2O$	Maine	2
rockbridgeite	(Fe ²⁺ , Mn ²⁺)Fe ₄ ³⁺ (PO ₄) ₃ (OH) ₅ ?	South Dakota	3
sabugalite	$HAl(UO_2)_4(PO_4)_4 \cdot 6H_2O$	France	12
sarcopside	(Fe ²⁺ , Mn ²⁺ , Ca) ₇ (PO ₄) ₄ F ₂	New Hampshire	8
scorzalite	$(Fe^{2+}, Mg)Al_2(PO_4)_2(OH)_2$	South Dakota	3
sicklerite	(Li, Mn ²⁺ , Fe ³⁺)(PO ₄)	South Dakota	3
strengite	$Fe^{3+}(PO_4) \cdot 2H_2O$	Germany	6
svanbergite	$SrAl_3(PO_4)(SO_4)(OH)_6$	Nevada	4
torbernite	$Cu(UO_2)_2(PO_4)_2 \cdot 8-12H_2O$	Mexico	1
triplite	(Mn ²⁺ , Fe ²⁺ , Mg, Ca) ₂ (PO ₄)(F, OH)	South Dakota	3
triploidite	(Mn ²⁺ , Fe ²⁺) ₂ (PO ₄)(OH)	Connecticut	2
tryphylite	Li(Fe ²⁺ , Mn ²⁺)(PO ₄)	South Dakota	1
turquois	$CuAl_6(PO_4)_4(OH)_8 \cdot 4H_2O$	Mexico	3
variscite	$Al(PO_4) \cdot 2H_2O$	Nevada	1
vashegyite	$Al_4(PO_4)_3(OH)_3 \cdot nH_2O?$	Hungary	6
vauxite	$Fe^{2+}Al_2(PO_4)_2(OH)_2 \cdot 7H_2O$	Bolivia	3
vivianite	$Fe_{3}^{2+}(PO_{4})_{2} \cdot 8H_{2}O$	Idaho	3
wagnerite	$Mg_2(PO_4)F$	Austria	5
wavellite	$Al_3(OH)_3(PO_4)_2 \cdot 5H_2O$	Arkansas	1
weinschenkite	$(Y, Er)(PO_4) \cdot 2H_2O$	Germany	12
wilkeite	Ca ₁₀ O((Si, P, S,) O ₄) ₆	California	7
wolfeite	(Fe ²⁺ , Mn ²⁺) ₂ (PO ₄)(OH)	New Hampshire	2
woodhouseite	$CaAl_3(PO_4)(SO_4)(OH)_6$	California	4
xanthoxenite	$Ca_2Fe(PO_4)_2(OH) \cdot 1\frac{1}{2}H_2O$	New Hampshire	6
xenotime	$Y(PO_4)$	Norway	11

TABLE 2—(Continued)

¹ For verification, x-ray diffraction, physical and optical data were compared with data from Palache, Berman and Frondel (1956), Larsen and Berman (1934), and the ASTM Powder Data Card File.

 2 Formulas are those given in Palache, Berman and Frondel (1951) and on the ASTM cards.

- ⁸ 1. Wards Natural Science Establishment, Rochester, New York.
 - 2. Smithsonian Institution, Washington, D.C.
 - 3. W. L. Roberts, Rapid City, South Dakota.
 - 4. Geoproducts International, Inc., Albany, California.
 - 5. Southwest Scientific, Scottsdale, Arizona.
 - 6. Minerals Unlimited, Berkeley, California.
 - 7. A. W. Woodford, Pomona College.
 - 8. D. Jerome Fisher, Univ. of Chicago.
 - 9. E. C. Moreno, TVA, Alabama.
- 10. Miss Barbara Lowe, Washington State University.
- 11. C. E. Ricketts, Washington State University.
- 12. Camden Mineral Co., Camden, Maine.

actinolite	epidote	microcline
asbestos	garnet	pyrite
augite	gibbsite	quartz
barite	gypsum	siderite
biotite	hornblende	titanite
chlorite	limonite	tourmaline
corundum	magnetite	zircon

TABLE 3. MINERALS THAT GAVE A NEGATIVE TEST WITH THE STRUCHNINE-MOLYBDATE REAGENT

A consideration of possible interferences in the molybednum blue method for phosphorus (Feigl, 1954; Boltz, 1958) suggested that silica, arsenic, fluoride, tungsten, and tin might interfere. It is obvious from the test of minerals common to soils and sediments, that under the conditions of this test silicates do not interfere. Realgar (As²⁻), fluorite (F^{-}), wolframite (WO₄²⁻), and cassiterite (Sn⁴⁺), did not give a positive test. However, arsenic in the form of arsenate (from reagent KH₂AsO₄) did give a positive test.

DISCUSSION

Two essentially different types of reactions were investigated as to the practicability of their use as an aid in the isolation of individual phosphate grains. The use of organic dyes, depends upon staining or otherwise causing a change in color of the grains themselves. Some dyes stained only a portion of the mineral sample. No attempt was made to determine why this differential staining occurred. It is not known whether it was the pure mineral that stained, an impurity, or merely a unique physical state of the grain surface. However, in the case of the synthetic variscite sample, X raying indicated that the stained part of the sample was an impurity. It is quite likely that with the proper selection of dyes one would be able to differentiate the components of many impure phosphate samples.

The use of a colored complex depends upon the partial dissolution of the grain, a disadvantage for relatively soluble phosphates. The great potential advantage of this approach is its relative specificity for phosphates. Based upon tests of standard minerals, the procedure devised in this investigation appears to preserve this advantage to a remarkable degree.

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