Hayes and Picker tubes. In all cases, copper targets with nickel filters were operated at 40 kv. and 15 ma.

Results and Discussion

An analysis of the powder patterns obtained[7] gave the d spacings summarized in Table I as characteristic of the gallates formed, other lines of low

TABLE I

SUMMARY OF d VALUES FOR GALLATES

Mg-Ga_2O_4	Cu-Ga_2O_4	Zn-Ga_2O_4	Cd-Ga_2O_4	La-GaO_3	Sr-Ga_2O_4	Ba-Ga_2O_4
4.78	4.81	4.83	4.96	2.73[a]	2.86[a]	3.15[a]
2.93[d]	2.94[d]	2.96[d]	3.05[d]	1.93[c]	2.02[c]	2.68[b]
2.50[a]	2.51[a]	2.52[a]	2.59[a]	1.58[b]	1.65[b]	2.17[f]
2.07	2.08	2.09	2.15	1.37	1.43	2.04[d]
1.69	1.70	1.71	1.77	1.22	1.28	1.97[f]
1.60[c]	1.60[c]	1.60[c]	1.66[c]	1.12	1.17	1.82[f]
1.47[b]	1.47[b]	1.48[b]	1.52[b]	1.03	1.08	1.70[e]
1.32	1.32	1.33	1.37	0.966	1.01	1.68[e]
1.25	1.25	1.26	1.30	0.865	0.952	1.63[e]
1.20	1.20	1.21	1.24		.903	1.55[e]
1.11	1.11	1.12	1.15		.862	1.50[f]
1.08	1.08	1.09	1.12		.824	1.44[f]
1.04	1.04	1.04	1.07		.791	1.28[f]
						1.24[f]

[a] First intensity. [b] Second intensity. [c] Third intensity. [d] Fourth intensity. [e] Weak (W). [f] Very weak (VW).

intensity in the patterns being those of small quantities of unreacted starting materials. Spinel formation is indicated with copper(II), magnesium, zinc and cadmium, results for the last three agreeing well with those previously reported.[3-6] Data for the lanthanum compound, $LaGaO_3$, indicate a perovskite type of structure, a structure characteristic of the comparable compounds $LaFeO_3$, $LaMnO_3$ and $LaCrO_3$.[8] The perovskite structure appears probable for the strontium compound as well (compare SrV_2O_4[9]), but the data for the barium compound are completely different and do not indicate a cubic structure by the "slide-rule test." An apparently hexagonal structure is tentatively assigned to this material. No evidences of gallate formation were obtained with calcium materials.

Unit cell lengths (a_0) for the spinel and perovskite structures are summarized in Table II.

TABLE II

HIGH TEMPERATURE GALLATE(III) FORMATION

Material sintered with Ga_2O_3	Sintering conditions, hr.	°C.	Results from powder patterns	a_0, Å.	Cation radius Å.
MgO	5	1250	$MgGa_2O_4$:spinel	8.29 ± 0.05	0.65
CuO	22	900	$CuGa_2O_4$:spinel	8.31 ± .05	.70
ZnO	22	900	$ZnGa_2O_4$:spinel	8.37 ± .05	.74
CdO	20	900	$CdGa_2O_4$:spinel	8.59 ± .05	.97
$Sr(NO_3)_2$·$4H_2O$	12	850	$SrGa_2O_4$:perovskite(?)	*ca.* 4.04	1.13
	20	900			
$Ba(NO_3)_2$	12	850	$BaGa_2O_4$:hexagonal(?)		1.35
	20	900			
La_2O_3	7	1250	$LaGaO_3$:perovskite	3.86 ± 0.05	1.15

Where other data are available for comparison, agreement is excellent. It is apparent that unit cell length varies directly with dipositive cation radius in the spinels. Increase in cation radius beyond a certain value destroys the spinel structure. From the data presented here, it appears that spinel-like gallates can result only when this radius is below 1.00 Å. Increase in cation radius beyond this limit gives first the perovskite (also cubic) and then a non-cubic arrangement. Cation radius is not, of course, the only structure-determining factor, but it appears to be of importance.

Although high basicity in general promotes reaction between a metal oxide and gallium(III) oxide, factors such as surface effects, previous treatment and volatility may nullify this trend. Thus, cadmium oxide reacts readily at 900°, but magnesium oxide reacts scarcely at all even on sintering for 72 hr. at 1000°. This is in agreement with the observations of Hauptmann and Novák[3] and is paralleled by similar observations on the magnesium oxide–indium(III) oxide system.[10]

Acknowledgment.—Support received from the Office of Naval Research is gratefully acknowledged.

(7) G. L. King, Doctoral Dissertation, University of Illinois, 1949.

(8) A. J. Wells, "Structural Inorganic Chemistry," 2nd Ed., Clarendon Press, Oxford, 1950, pp. 379–382.

(9) W. Rüdorff and B. Reuter, *Z. anorg. Chem.*, **253**, 177 (1947).

(10) T. Moeller and J. G. Schnizlein, *J. Phys. Chem.*, **51**, 771 (1947).

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The Diffusion Coefficient and Molecular Weight of Alkaline Phosphatase

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In 1935, Albers and Albers[1] reported diffusion experiments on alkaline phosphatase from swine kidneys, from which they calculated the molecular weight of the enzyme to be 6,000 to 10,000. This is an exceptionally low value for the molecular weight of an enzyme. Kraemer, *et al.*,[2] observed that serum alkaline phosphatase was associated with serum globulins in the ultracentrifuge. Hence a redetermination of the diffusion coefficient of the enzyme appeared of value.

Experimental

Diffusion coefficient determinations were carried out using the diaphragm cell method of Anson and Northrop,[3] as described by Northrop, *et al.*[4] Two cells were used having capacities of 42.4 and 47.7 ml., respectively. They were fabricated with Corning grade "F" fritted glass discs. The cells were calibrated with 2 *N* NaCl at both 5.0 and 25.0° in the manner described by Northrop.[4] The cell constants of 0.301 and 0.272, respectively, were repeatedly redetermined over the two-year period of use and the values obtained were constant within ±0.3%. Only differential diffusion coefficients were determined. Each determination with enzyme consisted of 4 to 5 consecutive 24-hour diffusion intervals. Constancy in the rate of diffusion was usually observed after 48 hours, indicating adequate equilibration of the membrane after this time.

Enzyme assays and preparations were made as previously

(1) H. Albers and E. Albers, *Z. physiol. Chem.*, **232**, 165, 189 (1935).

(2) E. O. Kraemer, L. Weil, E. B. Sanigar and M. T. Allen, *J. Franklin Institute*, **232**, 587 (1941).

(3) M. L. Anson and J. H. Northrop, *J. Gen. Physiol.*, **20**, 575 (1937).

(4) J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," 2nd edition, Columbia University Press, New York, N. Y., 1948.

TABLE I

DIFFUSION OF ALKALINE PHOSPHATASE AT 5°

Cell no.	pH, 25°	Solvent	Enzyme: Source	Enzyme: Concentration, P.U./ml.	Enzyme: Specific activity, P.U./mg./P.N.	Diffusion coefficient, cm.² per day: Protein	Diffusion coefficient, cm.² per day: Enzyme
3	7.2	0.1 ionic strength phosphate buffer	Albers' horse kidney preparation[a]	584	250 approx.	0.0348	0.0238
3	6.9	0.1 *M* phosphate buffer also 0.5 *M* NaCl	Albers' horse kidney preparation	602			.0218
3	7.26	0.1 ionic strength phosphate buffer	Swine kidney[b]	1540	600		.0221
3	9.02	0.2 *M* veronal also 0.01 *M* DL-alanine	Swine kidney	1600	600		.0200
4	9.04	0.2 *M* veronal also 0.01 *M* DL-alanine	Swine kidney	1180	600		.0190
3	7.2	0.1 ionic strength phosphate buffer	Calf intestinal mucosa (Armour's)	120	111		.0161
3	7.2	0.1 ionic strength phosphate buffer	Albers' sheep kidney preparation[a]	178	77		.0180
3	7.2	0.1 ionic strength phosphate buffer	Swine kidney[b]	2800	2500	.0236	.0170
3	7.2	0.1 ionic strength phosphate buffer	Swine kidney	3230	1050	.0257	.0178
3	7.2	0.1 ionic strength phosphate buffer	Swine kidney	756	1050		.0163

[a] Ref. 1. [b] Ref. 5, 7.

described.[5,6,7] Swine kidney alkaline phosphatase preparations employed in this investigation are among the purest that have been reported. Protein was determined colorimetrically by the procedure of Lowry, *et al.*,[8] or by Kjeldahl.[9] Improved accuracy of the colorimetric determination of protein appeared to be obtained by carrying out the entire procedure at 25°. Twice crystallized ovalbumin was used as a standard protein. The concentrated ovalbumin solution was stored at −18° and contained 50% glycerol. Under these conditions it was adequately stable.

Diffusion coefficients for crystalline ovalbumin and crystalline swine pepsin were determined, giving values in accord with the literature.

Results and Discussion

Values obtained at 5° are compiled in Table I. Five additional experiments were carried out at 25° giving an average value of 0.038 cm.² per day. Molecular weight calculations from this latter figure are in fair agreement with those obtained at 5°. It can be seen that the diffusion coefficient ranges between 0.016 to 0.024 cm.² per day, irrespective of the solvent, *p*H, enzyme source, purity or concentration. In the last two experiments, in which the diffusion of protein was measured, it is apparent that protein is diffusing at a faster rate than enzyme, indicating the presence of impurities, but even so the values are in the same order of magnitude.

Molecular weight calculations from these data give values of 500,000 and over. Admittedly determinations in this molecular size range must be considered to be only approximations. Yet there is complete disagreement between our findings and those of Albers.[1] With this in mind, several experiments with commercial, graded porosity ultra-filters were carried out, and these, too, were indicative of a large molecular weight.

A point of interest is the similarity in results irrespective of the enzyme source. At least in this respect, it has not been possible to demonstrate significant differences between alkaline phosphatases from various sources.

The differential diffusion coefficient for several types of alkaline phosphatase has been determined using the diaphragm cell method. An average value of 0.0192 cm.² per day was obtained at 5°. Molecular size estimations from these data, making the usual assumptions as to shape and density, are from 500,000 to 1 million, with the best estimation being 800,000. No significant difference was detected with respect to this property, between intestinal and kidney alkaline phosphatase, the latter enzyme being obtained from three species.

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(5) J. C. Mathies, *Biochim. Biophys. Acta*, **7**, 387 (1951); *Science*, **115**, 144 (1952).

(6) J. C. Mathies, E. D. Goodman and L. Palm, *Am. J. Physiol.*, **168**, 352 (1952).

(7) J. C. Mathies and E. D. Goodman, *Federation Proc.*, **11**, 255 (1952); Abstracts 123rd Meeting Am. Chem. Soc., pp. 24C (1953).

(8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(9) A. Hiller, J. Plazin and D. D. Van Slyke, *J. Biol. Chem.*, **176**, 1401 (1948).

Restricted Coupling in a Substituted Phenol[1]

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As part of the preparation of 4-arsonophenylazo derivatives of various phenols as potential carcinolytic agents, the coupling of diazotized *p*-arsanilic acid with 4-(1,1,3,3-tetramethylbutyl)-phenol (also commonly called octylphenol[3] or diisobutylphenol) (I) was studied. It was found that even though both positions in the phenol ortho to the hydroxyl group were unsubstituted, only one 4-arsonophenylazo group could be introduced even when an excess of the diazonium salt was used. This was somewhat surprising since tyrosine[4] couples with two moles of diazonium salt quite readily. Other 4-substituted phenols[5] also have shown coupling in both ortho positions.

It was necessary to add ethyl alcohol to the medium to keep the octylphenol in solution in alkali for the coupling reaction and it was thought that possibly the alcohol might have caused decomposition of enough of the diazonium salt to prevent coupling in both ortho positions. However, no phenylarsonic acid, the expected product from such decomposition, could be isolated from the reaction mixture. Furthermore, when the mono-substi-

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(3) Kindly furnished by Rohm and Haas Co.

(4) H. Pauly, *Z. physiol. Chem.*, **94**, 284 (1915).

(5) L. Pauling, *et al.*, THIS JOURNAL, **64**, 2994 (1942).