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Chemical parameters in the study of in vivo and in vitro interactions of transition elements[1]

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As THE STUDY OF trace element metabolism has advanced in recent years nutritionists have become aware of many biological interactions among these nutrients. For instance, it has been reported that high levels of dietary manganese decreased hemoglobin synthesis and iron stores and that this effect could be overcome by feeding supplemental iron (2, 5, 8). High levels of zinc in the diet produced an anemia which could be corrected by the addition of copper to the diet (1, 9, 12). Another example of such a relationship is the finding that selenium toxicity could be overcome by the addition of arsenic to the drinking water (6, 7). Supplee (10) reported that low levels of cadmium decreased growth and increased hock and feather abnormalities in turkey poults and that these symptoms could be overcome by the addition of zinc to the diet. High levels of tungstate have been shown to inhibit growth and xanthine oxidase levels in chicks and rats and these effects could be reversed by the addition of molybdenum to the diet (4). Many other examples of interrelationships of various kinds among the elements have been demonstrated but these particular examples have been chosen for the purpose of illustrating the thesis underlying the work to be presented in this report. The thesis may be stated simply as, "Those elements whose physical and chemical properties are similar will act antagonistically to each other biologically."

The physical and chemical properties of elements depend to a large extent on their electronic structure. Schrödinger has suggested that the movement of electrons about a nucleus may be expressed in terms of wave equations, the equation for a single electron being:

$$\frac{d^2f}{dx^2}+\frac{d^2f}{dy^2}+\frac{d^2f}{dz^2}+\frac{8\pi^2m}{h^2}(W-V)f=0$$

The wave function is denoted as f, m is the mass of the electron, W is the total energy, V is the potential energy, and h is Planck's constant. This one electron wave function can be written as a product of a function of the radial distance of the electron from the nucleus with another function which is dependent only on the angular coordinates of the electron. The angular functions are independent of the element; there is just one angular distribution of s electrons, three for p electrons, and five for d electrons. These distributions are commonly pictured as in Fig. 1, but it should be remembered that these are, in fact, three dimensional. The two important points to keep in mind are that much of the chemistry with which we are concerned is a reflection of the angular distribution of the electronic orbitals, and that the angular distribution of the electronic orbitals is independent of the element studied.

The orbitals of an element exist in an orderly arrangement. By convention these are called, in order of increasing distance from the nucleus, 1s, 2s, 2p, 3s, 3p, 3d, 4s, 4p, 4d, 4f, 5s, 5p, 5d, 5f, 5g, and so forth. Our concern in the work to be reported is with the s, p, and d orbitals. Each orbital may have a maximum of two electrons and these must be of opposite spin states.

The chemistry of the elements in which we are interested is coordination chemistry, which deals with the attachment of molecules, ligands, to the central elemental atom. The number of ligands may be two, four, six, or eight per central atom and has no necessary relationship to the valency of the element. Valency does have a role to play, however, at least in our thinking, since we are concerned with ions and not the elemental atom.

Our first experimental work arose from our interest in copper metabolism. Copper may exist in either the monovalent or divalent form and some of its biological importance is related to its ability to oscillate between the cuprous and cupric state, i.e., the ability to accept and donate electrons. The orbital arrangement of the outermost electrons of the cupric and cuprous ions may be depicted as in Fig. 2. The 1s, 2s, 2p, 3s, and 3p orbitals are filled with two electrons each. The 3d orbitals of the cuprous ion are also filled, the arrows in the figure representing electrons and the direction of the arrows the spin state. The cupric ion has one less electron and it happens because of energy considerations that one 3d electron is promoted to a 4p orbital, leaving a 3d orbital

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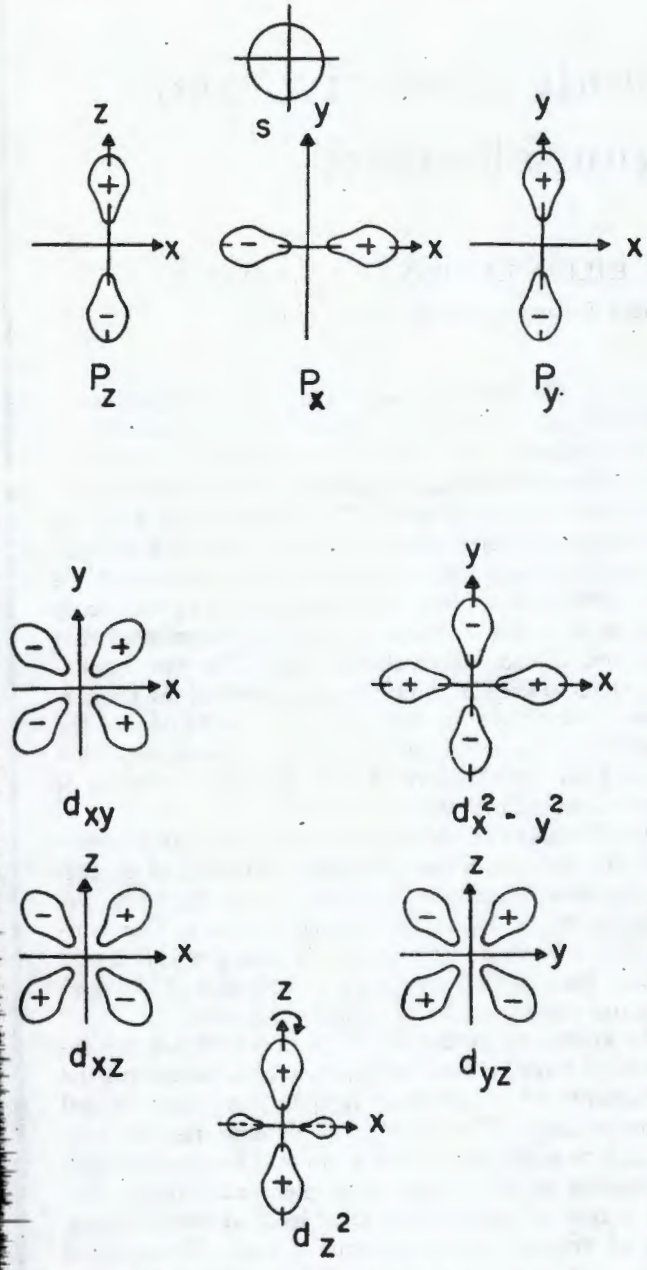

FIG. 1. Diagrammatic illustration of electron orbitals.

as well as the 4*s* orbital without electrons and available for bond formation. The *p* orbitals tend to be filled in complex formation since the complex then would have the structure of the inert, and therefore, most stable krypton.

The four empty orbitals indicate that both cuprous and cupric ions should coordinate with four ligands. The structure of the complexes formed in each case, however, will be different. In cuprous complexes the structure is tetrahedral, while the cupric complexes will tend to have a square planar structure. In the former, the four ligands approach the central atom along bisectors of the cartesian coordinates, while in the square planar complexes the ligands may be thought of as approaching the central atom along the *x* and *y* axes.

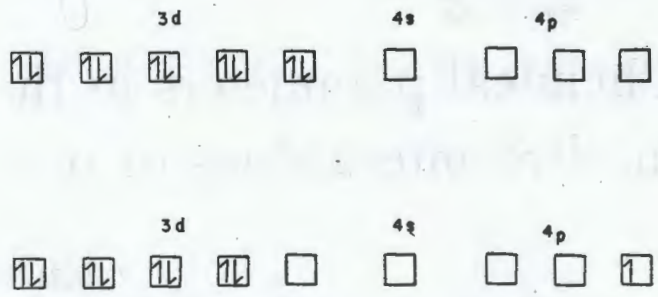

FIG. 2. Orbital arrangement of cuprous ions (top) and a cupric ion (bottom).

TABLE 1. *Basic dried skim milk-glucose diet*

Ingredients	Amount
	%
Glucose[a]	32.5
Dried skim milk	60.0
Vegetable oil[b]	5.0
DL-Methionine	0.3
L-Arginine·HCl	0.5
Glycine	0.5
NaCl	0.5
$MgSO_4 \cdot 7H_2O$	0.5
	mg/kg
$FeSO_4 \cdot 7H_2O$	528
$MnSO_4 \cdot H_2O$	220
$CuSO_4 \cdot 5H_2O$	100
Choline chloride	1,540
Vitamin mixture[c]	1,980

[a] Cerelose from Corn Products Refining Company, New York, N. Y. [b] Wesson oil from The Wesson Oil Company, New Orleans, La. [c] Supplies the following vitamins per kg of diet: (in mg) thiamin, 4.0; riboflavin, 7.2; folic acid, 1.8; DL-α-tocopheryl acetate, 22.0; menadione sodium bisulfite, 1.4; pantothenic acid, 22; niacin, 140; pyridoxine, 6.0; vitamin B_{12}, 0.006; vitamin A, 2,000 USP units; vitamin D, 200 ICU.

TABLE 2. *Interaction of zinc and copper on hemoglobin concentration, mortality, and body weight of chicks*

Zinc, ppm	Copper, ppm					
	0	10	0	10	0	10
	Hb, g/100 ml		% Mortality		Wt, g	
0	6.5	8.5	8.7	0	262	272
50	5.9	8.0	18.2	4.5	205	320
100	6.0	8.8	52.0	7.7	182	294
200	4.8	8.0	70.8	20.0	139	291
300	4.1	6.6	88.0	4.2	127	310

The electronic structure of the cuprous and cupric ions is not unique. The Zn^{2+}, Cd^{2+}, and Hg^{2+} ions all have the same electronic structure of the valence shell as the cuprous ion, while the Ag^{2+} ion has the same structure as the cupric ion. If the thesis which we wished to examine were valid, all of these ions should be antagonistic to copper.

The basal diet used in these experiments was com-

posed of dried skim milk, glucose, vitamins, amino acids, and the required minerals except for copper. The composition of the diet is presented in Table 1. It contains less than 1 ppm copper and about 25 ppm zinc. In the first experiment zinc was added as the sulfate, the control chicks received cupric sulfate to supply the indicated levels of this element.

When the levels of zinc added to the basal diet were varied from 0 to 300 ppm, the results presented in Table 2 were obtained. Two hundred parts per million zinc depressed hemoglobin concentration in the copper-deficient but not the control chicks, while at 300 ppm the hemoglobin levels of both groups were depressed. Zinc at a level of 100 ppm resulted in a significant increase in mortality in the copper-deficient, but not in the control chicks. As little as 50 ppm zinc reduced growth in the copper-deficient, but again not in the control chicks. These studies all point to the fact that zinc can act as a copper antagonist, that is, increase the severity of copper deficiency, and is in accord with the work of Smith and Larson (9) using rats fed much higher levels of zinc in a diet that was not copper deficient.

We next turned our attention to the possible interaction of cadmium with copper. At this point we were confronted with a complication. If the electronic structure of cadmium is the same as the cuprous ion it is also the same as the zinc ion. Since both copper and zinc are essential elements, we could expect a double interaction, cadmium with zinc as well as cadmium with copper. To unravel this aspect of the problem a factorial experiment was conducted in the presence and absence of addition of all three elements (Table 3).

TABLE 3. *Interaction of cadmium with zinc and copper*

Cd, ppm	Zn, ppm	Cu, ppm 0	Cu, ppm 10	Significant Effects
		Body wt, g		
0	0	214	246	Cu[b], Cd[b]
100	0	131	163	and (Zn × Cd)[b]
0	200	174	222	
100	200	149	187	
		Mortality[a]		
0	0	4	1	Cu[b], Cd[b]
100	0	17	1	(Cd × Cu)[b]
0	200	13	0	
100	200	19	1	

[a] No. out of 20 chicks at 19 days.
[b] $P < 0.01$.

Copper significantly increased the weights of the chicks and cadmium significantly decreased them. Zinc had no overall effect alone. There was a significant zinc-cadmium interaction indicating that in the presence of zinc, cadmium was much less effective in reducing growth. There was no copper-cadmium interaction indicating that the effect of cadmium on growth was independent of the level of copper fed.

The mortality was significantly less in those lots receiving copper and significantly more in those receiving cadmium. In this instance there was a significant interaction between cadmium and copper which is indicative of the fact that cadmium increased mortality in the absence but not in the presence of copper. These data, then, show two interactions which are in accord with the hypothesis underlying these experiments. Cadmium is antagonistic to zinc in accord with the data reported by Supplee using turkey poults, and cadmium is also antagonistic to copper.

We next turned our attention to the effect of silver on copper metabolism. Since the Ag^{2+} ion is like the cupric ion and unlike the cuprous and zinc ions, if our hypothesis was valid the effect of the silver ion should be completely counterbalanced by copper (Table 4). Silver at 100 ppm reduced growth in the copper-deficient but not in the control chicks. At 50 ppm mortality was increased in the copper-deficient, but not in those groups receiving copper. As little as 10 ppm silver reduced the hemoglobin concentration in the deficient but not in the control chicks. The elastin content of the aorta, which is sensitive to the copper content of the diet, was also reduced by the addition of 10 ppm silver to the diet of the deficient chicks and this effect, too, was completely overcome by the addition of copper to the diet.

In this series of measurements, then, silver acted as a

TABLE 4. *In vivo interaction between silver and copper*

Silver, ppm	Copper, ppm 0	10	25	0	10	25	0	10	25	0	10	25
	3-week body wt[a] g			Mortality 4 wk[a]			Hb[b] g/100 cm³			Aortic elastin[c] % wet wt		
0	145	196	232	5	2	3	7.45	7.17	7.72	7.58	11.63	12.12
10	125	190	229	5	1	0	6.37	7.32	9.00	5.39	11.68	10.46
25	139	199	244	3	0	2	6.05	7.35	8.22	4.92	14.58	12.45
50	126	240	243	10	2	0	5.02	7.40	7.30	6.67	12.05	11.15
100	110	174	240	12	1	4	4.62	7.00	7.60	5.26	11.03	11.33
200	96	187	207	13	0	0	3.52	7.75	8.32	4.78	11.59	12.46

[a] Twenty chicks started in each treatment. [b] Mean of 4 determinations in each treatment. [c] Mean of 5 determinations in each treatment.

TABLE 5. *Interactions between copper, mercury, zinc, and cadmium*[a]

		Avg wt at 2 weeks of age, g			
		Cu, 0		Cu, 25 ppm	
		Hg, 0 g	Hg, 400 ppm g	Hg, 0 g	Hg, 400 ppm g
Cd, 0	Zn, 0	144	123	160	138
	Zn, 400 ppm	87	108	147	138
Cd, 100 ppm	Zn, 0	82	81	100	78
	Zn, 400 ppm	87	100	115	91

[a] Twenty chicks per treatment.

TABLE 6. *Interactions of copper with mercury and zinc*

		Copper, ppm	
		0	25
		Body wt, g 2 weeks	
Mercury, ppm	0	100	130
	400	104	111
Zinc, ppm	0	107	119
	400	95	123

copper antagonist, and, since the effects of silver were completely overcome by the addition of copper alone, there was evidently no antagonism with zinc.

In the study of the effect of mercury on copper metabolism, the experiment was expanded to include possible mercury interactions with zinc and cadmium as well as copper, that is, a four-way factorial experiment was conducted (Table 5). The analysis of variance of this experiment indicated that there was a significant interaction between mercury and copper (Table 6). The growth of chicks was not depressed by the addition of mercury to the copper-deficient diet. On the other hand, mercury did depress the growth of those chicks receiving the copper-supplemented diet. This is in contrast to the interaction of zinc and copper in this experiment and the experiment reported previously. Zinc depressed growth in the absence but not the presence of copper. As the experiment progressed and the mortality pattern emerged much the same relationships were observed. The mortality at 4 weeks when the experiment was terminated is presented in Table 7. In the absence of copper, mercury, in contrast to zinc and cadmium, did not cause mortality; however, when copper was present there was mortality among the mercury-fed animals. By these two criteria, then, growth and mortality, there is a mercury-copper interaction but it is not one of antagonism since, when the copper content of the diet was low and the antagonism could be expressed most fully, mercury had no effect.

The reason for the different behavior of mercury is not known exactly but it may well be related to the fact that the orbitals of mercury which we are considering are 6*s* and 6*p* and thus are removed much further from the nucleus than the 4*s* and 4*p* orbitals of the cuprous ion. The wave function of the 6*s* electrons is less than that of the 4*s* electrons because of the increased distance from the nucleus, and, therefore, the reactivity of the 6*s* electrons is much less than that of 4*s* electrons. The 6*s* orbitals have been found empirically to be relatively inert. Furthermore, mercury has been found to coordinate with two ligands linearly and form polymeric structures.

The data presented up to this point may be summarized by saying that zinc, cadmium, and silver acted as copper antagonists, and that cadmium acted as a zinc antagonist and that these findings are in accord with the hypothesis that elements whose physical and chemical properties are near enough alike will tend to be antagonistic to each other. The mercuric ion, whose electronic structure is similar to the cuprous ion, did not act as a copper antagonist, but this may have been a reflection of the relative inertness of the 6*s* orbital.

To determine whether or not the hypothesis under consideration was more generally valid, we turned our attention to another section of the periodic table, that containing selenium. The Se^{4+} ion has the electronic structure shown in Fig. 3. The 4*p* and 4*d* orbitals of the ion are without electrons. This is the same structure that the As^{3+}, Sn^{2+}, and the Te^{4+} ions assume except that the orbitals of the latter two are 5*s*, 5*p*, and 5*d*. If the hypothesis under examination were valid these ions should show some mutual antagonisms. To determine whether or not this was true experiments were conducted measuring the effect of arsenic, tellurium, and tin on the absorption of selenium.

In these studies an isolated segment of the chick intestine was used. The chicks were anesthetized and the intestine exposed surgically and a segment tied off. A solution containing ^{75}Se along with unlabeled selenium was in-

TABLE 7. *Effect of mercury, zinc, and cadmium on mortality of chicks*

Supplement	Cu, ppm	
	0	25
	No. dead[a]	
None	3	0
Zn, 400 ppm	19	3
Cd, 100 ppm	19	5
Hg, 400 ppm	1	7
Zn, 400 + Cd, 100 ppm	20	2
Cd, 100 + Hg, 400 ppm	20	12
Zn, 400 + Hg, 400 ppm	14	3
Hg, 400 + Cd, 100 + Zn, 400 ppm	19	8

[a] Twenty chicks started.

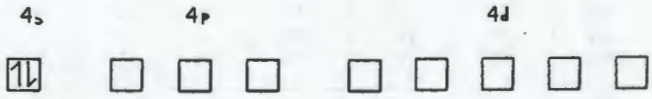

FIG. 3. Orbital arrangement of Se^{4+}, As^{3+}, Sn^{2+}, and Te^{4+} ions.

TABLE 8. *Effect of arsenic, tin, and tellurium on the absorption of selenium*

	% Absorbed		
	Exp 1	Exp 2	Exp 3
Se 0.02 μmole	46.2	61.1	
Se 1.0 μmole	52.6	72.2	69.1
Se 0.02 μmole + As 1 μmole	28.1		
Se 1.0 μmole + As 1.0 μmole	25.6		
Se 0.02 μmole + Te 1.0 μmole		36.6	
Se 1.0 μmole + Sn 0.25 μmole			24.4

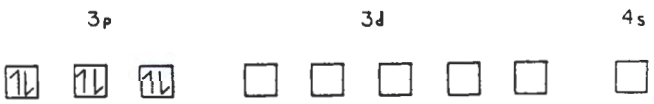

FIG. 4. Orbital arrangement of V^{5+} and Cr^{6+} ions.

troduced into the segment. The substances to be investigated, arsenic, tellurium, and tin, were also injected into the segment. The radioactivity of the selenium administered was determined with an automatic gamma scintillation counter. After 2 hours the chicks were killed and the intestinal segment removed and the radioactivity remaining determined. That radioactivity which had disappeared was considered absorbed (Table 8).

In the studies with arsenic and tellurium two levels of selenium were used to assure that any effect observed might not be caused by a simple overloading of the system with a metal. The increased level of selenium did not result in a decrease in absorption. The addition of arsenic, tellurium, or tin, however, markedly depressed the absorption of selenium. These results are, then, also in accord with the hypothesis that like ions are antagonistic biologically, in this instance at the absorptive level.

To further test the hypothesis we turned our attention to an examination of the effects of vanadium on chicks. This element is quite toxic at dietary levels of 25 ppm or less and has been found to uncouple oxidative phosphorylation both in vivo and in vitro (3). The element whose ions most closely resemble vanadium is chromium. The orbital arrangement of the V^{5+} and the Cr^{6+} ions is shown in Fig. 4.

To determine whether or not there was an interaction between chromium and vanadium, these two elements were incorporated into the diet of chicks (Table 9). Vanadium at a level of 20 ppm depressed growth and resulted in heavy mortality. The addition of chromium to the diet significantly decreased the magnitude of these responses to vanadium. In another experiment the interaction of dietary vanadium and chromium on oxidative phosphorylation was investigated (Table 10). As the dietary level of vanadium was increased oxidative phosphorylation was progressively uncoupled. The presence of chromium in the diet partially prevented the uncoupling. In an in vitro experiment in which vanadate and chromate were added directly to the liver mitochondrial suspensions, the results presented in Table 11 were obtained. Vanadate uncoupled oxidative phosphorylation and this effect was partially overcome by the simultaneous addition of chromate to the system.

By the criteria of growth, mortality, and uncoupling of oxidative phosphorylation, these data lend support to the hypothesis under investigation.

The work with oxidative phosphorylation brought to mind another element which has been known for many

TABLE 9. *Effects of dietary vanadium and chromium on growth and mortality of chicks*[a]

Chromium, ppm	Vanadium 0	Vanadium 20 ppm
Body wt, g at 3 weeks of age[b]		
0	238.1	98.5
500	256.6	125.2
1,000	232.6	158.9
2,000	182.7	193.4
Duncan's multiple range test:[c]		
98.5 125.2 158.9 182.7	193.4 232.6 238.1 256.6	
% mortality at 3 weeks of age		
0	6.7	86.6
500	6.7	66.7
1,000	10.0	40.0
2,000	6.7	13.3
Duncan's multiple range test:[c]		
6.7 6.7 6.7 10.0 13.3	40.0 66.7 86.6	

[a] Chicks were fed the dried skim milk diet in Table 1 supplemented as indicated with NH_4VO_3 and/or $CrCl_3$. [b] The values listed are averages of from 5 to 15 chicks. [c] Any two values underscored by the same line are not significantly different; any two not underscored by the same line are significantly different; $P = 0.05$.

TABLE 10. *Effect of dietary vanadium and chromium on the P/O ratio in chicken liver mitochondria*[a]

Vanadium, ppm	Chromium 0	Chromium 100 ppm	Chromium 400 ppm
0	1.80[b]	1.78	1.75
10	1.09	1.29	1.59
20	0.65	1.03	1.30
Duncan's multiple range test:[c]			
0.65 1.03 1.09 1.29 1.30	1.59 1.75 1.78 1.80		

[a] Reaction medium and conditions for P/O measurements were as follows: 40 mM succinate; 20 mM phosphate; 5 mM ADP; 4 mM KF; 10–15 mg of mitochondrial protein/ml; pH 7.4; room temperature (24–28 C). [b] Each reported P/O ratio is the average of six individual determinations. [c] Any two values underscored by the same line are not significantly different; any two not underscored by the same line are significantly different; $P = 0.05$.

years to be an uncoupler, arsenate. The mechanism of uncoupling by arsenate is believed to reside in the fact that the arsenate ion is chemically and structurally much like the phosphate ion.

Indeed, when one examines the molecular orbital diagrams of the phosphate and arsenate ions (Fig. 5) great similarities are seen. Furthermore, if the vanadate ion and chromate ion molecular orbital diagrams are constructed it can be seen that these also resemble phosphate.

TABLE 11. *Interaction of vanadate and chromate on oxidative phosphorylation*[a]

Chromate, mM	Vanadate, mM 0	Vanadate, mM 1
	P/O[b]	
0	1.85	0.75
0.1	1.62	0.98
1.0	1.75	1.38
10	1.81	1.30

[a] Reaction conditions and media were the same as in Table 10. [b] Each reported P/O ratio is the average of six determinations.

It was possible that this was also the reason behind the uncoupling by vanadate. When one examines the molecular orbital structures of phosphate, vanadate, and arsenate (Fig. 5) it can be seen that they are similar. In all three the oxygen is attached to the central atom in an sp^3 arrangement and thus forms a tetrahedral structure. The arsenate anion is arranged exactly as the phosphate anion except that the relevant orbitals are 4*s*, 4*p*, and 4*d*, instead of 3*s*, 3*p*, and 3*d*. The vanadate anion is like the arsenate anion except that the πd bond is to a 3*d* orbital instead of a 4*d*. Furthermore, the chromate anion is like the vanadate except that there are two πd bonds instead of one. In examining the elements for an analogous couplet with arsenate, selenate was found to be arranged like arsenate except that it also had two πd bonds. If the interaction of selenate with arsenate was like that of chromate with vanadate, selenate should partially prevent the uncoupling of oxidative phosphorylation by arsenate. The results of an experiment to determine if this were so is presented in Table 12. The addition of arsenate to the incubation medium resulted in uncoupling of oxidative phosphorylation and this was partially prevented by the addition of selenate.

To gain further insight into this phenomenon studies

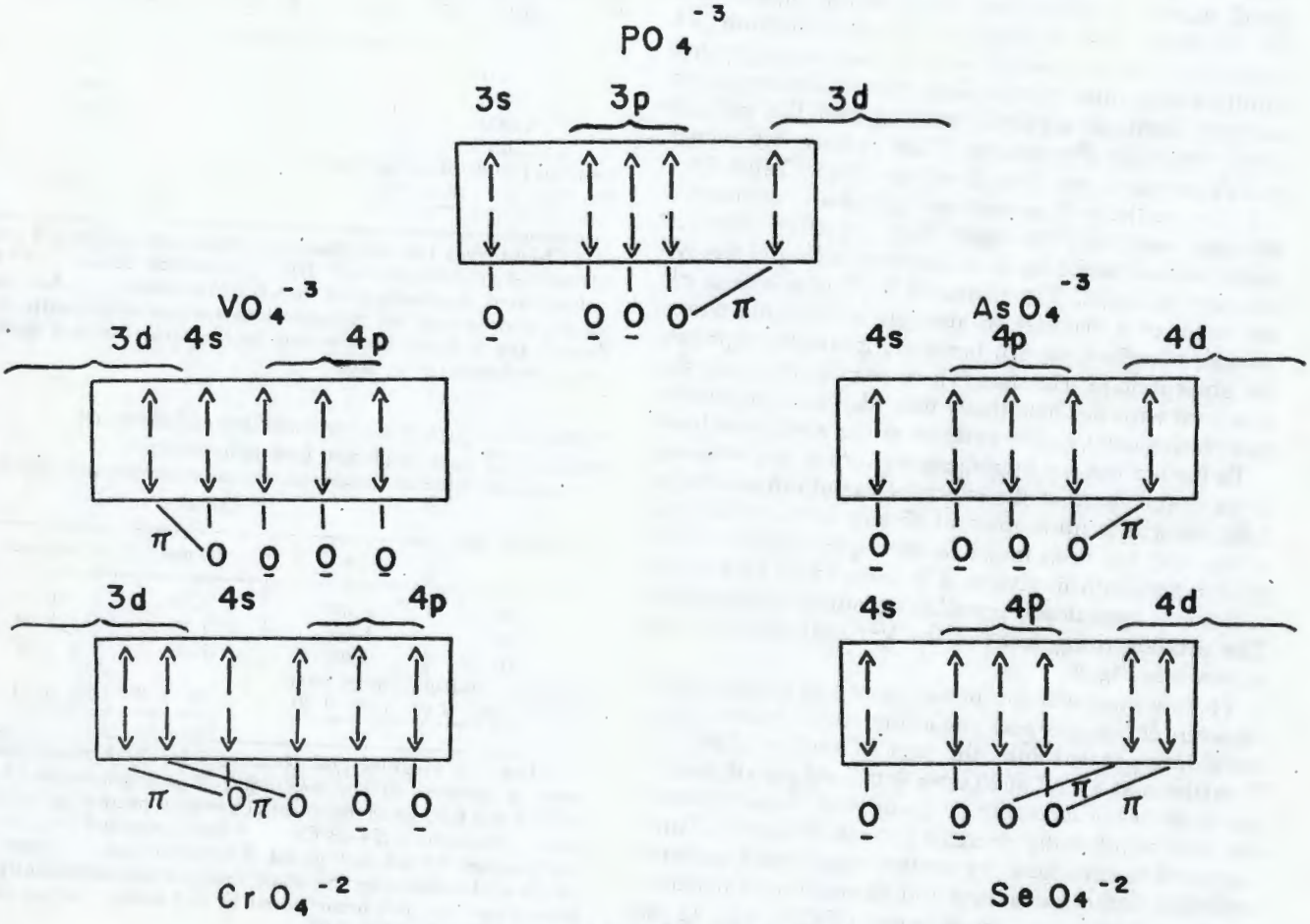

FIG. 5. Molecular orbitals of PO_4^{3-}, VO_4^{3-}, AsO_4^{3-}, CrO_4^{2-}, and SeO_4^{2-} anions.

TABLE 12. *Interaction of selenate and arsenate on oxidative phosphorylation*[a]

Selenate, mM	Arsenate, mM 0	Arsenate, mM 20
	P/O[b]	
0	1.72	0.59
2	1.68	1.26
20	1.56	1.46
50	1.58	1.30

[a] Reaction conditions and media were the same as in Table 10. [b] Each reported P/O ratio is the average of six determinations.

TABLE 13. *Effects of vanadate, arsenate, chromate, and selenate on the uptake of phosphate by respiring mitochondria*[a]

Treatment	cpm[b]
Control	15,925
1 mM VO_4	5,289[c]
40 mM AsO_4	9,680[c]
1 mM CrO_4	14,267
40 mM SeO_4	15,921

[a] Reaction media and conditions were the same as in Table 10, except that each contained 4 mM P_i (incl. 1 μCi ^{32}P/sample) with VO_4, AsO_4, CrO_4, or SeO_4 as indicated. [b] The counts per minute (cpm) reported are averages of five individual observations and represent the relative amounts of ^{32}P absorbed by the mitochondria. [c] Significant; $P = 0.01$.

TABLE 14. *Effects of high and low phosphate concentrations on the uptake of vanadate, arsenate, chromate, and selenate by respiring mitochondria*[a]

PO_4, mM	cpm[b]			
	1 mM $^{48}VO_4$	10 mM $^{76}AsO_4$	1 mM $^{51}CrO_4$	10 mM $^{75}SeO_4$
2	1,884	5,096	26,498	139,583
200	1,303	1,942	27,992	136,824
	M[c] = 0.804[e]	M = 0.594[e]	M = 0.298[f]	M = 0.051[d]

[a] Reaction media and conditions were the same as in Table 10, except as noted. [b] The counts per minute (cpm) reported are averages of six individual observations and represent the relative amounts of isotope absorbed by the mitochondria. [c] M is the substitute t value for comparing two means, according to P. G. Moore, 1957. [d] Not significant. [e] Significant; $P = 0.01$. [f] Significant; $P = 0.05$.

TABLE 16. *Effect of chromate on the uncoupling of oxidative phosphorylation by arsenate*

K_2CrO_4	P/O[a]	Chromate versus Arsenate[c] Analysis of Variance				
		Source	df	SS	MS	F
0	0.62	Treatments	3	0.0338	0.0113	2.54[b]
1 mM	0.67	Replications	5	0.0550	0.0110	
20 mM	0.57	Error	15	0.0668	0.00445	
50 mM	0.59	Total	23	0.1556		

[a] Each reported P/O ratio is the average of six individual determinations. [b] Not significant. [c] Reaction media and conditions were the same as in Table 10, except that the P_i concentration was 1 mM and each also contained 20 mM arsenate.

were conducted on the influence of the ion interactions on mitochondrial uptake. In the first experiment the uptake of phosphate by respiring mitochondria in the presence of the various anions was determined. In this experiment the incubation medium contained 4 mM of inorganic phosphate including 1 μCi of ^{32}P per sample (Table 13). Vanadate and arsenate inhibited the uptake of phosphate while chromate and selenate were without effect.

In the reciprocal experiment, the effect of phosphate on the uptake of vanadate, arsenate, chromate, and selenate, the results presented in Table 14 were obtained. When the phosphate level was increased from 2 to 200 mM, the uptake of vanadate and arsenate by the mitochondria was depressed. Increased phosphate did not depress the mitochondrial uptake of chromate or selenate, and, indeed, the uptake of chromate was slightly but significantly increased. These two studies indicate that the three anions which are most alike in their molecular structure are mutually antagonistic to each other as far as mitochondrial uptake is concerned.

In another experiment the interactions of chromate with vanadate and selenate with arsenate were examined (Table 15). Chromate and vanadate as well as selenate and arsenate are antagonistic to each other as far as mitochondrial uptake is concerned. Again, the sets of two anions which are most alike behave antagonistically.

To further examine the specificity of the interactions,

TABLE 15. *Effect of vanadate, chromate, arsenate, and selenate upon the uptake of ^{51}Cr, ^{48}V, ^{75}Se, and ^{76}As, respectively, by respiring mitochondria*[a]

$^{51}CrO_4$ vs. VO_4, 1 mM CrO_4 and VO_4 as indicated		$^{48}VO_4$ vs. CrO_4, 1 mM VO_4 and CrO_4 as indicated		$^{75}SeO_4$ vs. AsO_4, 2 mM P_i, 40 mM SeO_4 and AsO_4 as indicated		$^{76}AsO_4$ vs. SeO_4, 2 mM P_i, 40 mM AsO_4 and SeO_4 as indicated	
VO_4, mM	cpm[b]	CrO_4, mM	cpm	AsO_4, mM	cpm	SeO_4, mM	cpm
0	15,559	0	7,979	0	449,070	0	12,221
1	9,803	1	3,880	40	215,080	40	5,556
M[c] = 0.513[d]		M = 1.829[d]		M = 0.536[d]		M = 0.675[d]	

[a] Reaction media and conditions were the same as in Table 10, except as noted in this table; 1μ Ci of isotope was used per sample. [b] The counts per minute (cpm) reported are averages of six individual observations and represent the relative amounts of isotope absorbed by the mitochondria. [c] M is the substitute t value for comparing two means, according to P. G. Moore. *Biometrika* 44: 482, 1957. [d] Significant; $P = 0.05$.

an experiment was conducted in which oxidative phosphorylation was uncoupled by arsenate and levels of chromate were added (Table 16). The addition of chromate had no effect on the uncoupling action of arsenate indicating that in spite of the similarities between the selenate and chromate anions, i.e., the sp^3 tetrahedral configuration, the differences are great enough to prevent antagonistic interaction.

The results of all the studies presented in this report are in accord with the hypothesis originally proposed, that elements whose physical and chemical properties are enough alike, i.e., whose electronic structure is similar enough, will act antagonistically to each other biologically. The possible exception in this respect was that mercury did not act antagonistically to copper or zinc. However, the wave function does have a radial component and mercury was the only element tested which has two complete sets of orbitals, electron shells, different from the elements with which it was tested. It is possible that this difference in radius of the valence shell of electrons is restrictive as far as biological interaction is concerned; or the difference may be related to the tendency of mercury to form linear polymers instead of tetrahedral complexes.

The experiments on the interaction of phosphate, vanadate, arsenate, chromate, and selenate indicate that the interactions are not limited to cations but may also be extended to anions. The orbital structures of these anions are somewhat more complicated than the simple cations, but those that are most similar were antagonistic to each other and those that were the most dissimilar were not.

One final point should be made. In the studies reported here on the effect of arsenic, tellurium, and tin on the absorption of selenium, as well as those studies on the mitochondrial uptake of various anions, the site of biological interaction appears to be at the level of ion penetration of membranes. The work reported by Van Campen (11) on the effect of zinc and cadmium on the absorption of copper also indicates that the antagonistic effect among the various cations is at this physiological level. The results of the experiments suggest that when the ions are enough alike they will interfere with each other's uptake by tissues or organelle membranes.

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REFERENCES

1. Grant-Frost, D. R., and E. J. Underwood. *Australian J. Exptl. Biol. Med. Sci.* 36: 339, 1958.
2. Hartman, R. H., G. Matrone and G. H. Wise. *J. Nutr.* 57: 429, 1955.
3. Hathcock, J. N., C. H. Hill and S. B. Tove. *Can. J. Biochem.* 44: 983, 1966.
4. Higgins, E. S., D. A. Richert and W. W. Westerfield. *J. Nutr.* 59: 539, 1956.
5. Matrone, G., R. H. Hartman and A. J. Clawson. *J. Nutr.* 67: 309, 1959.
6. Moxon, A. L. *Science* 88: 81, 1938.
7. Moxon, A. L., and M. Rhian. *Physiol. Rev.* 23: 305, 1943.
8. Robinson, N. W., S. L. Hansard, D. M. Johns and G. L. Robertson. *J. Animal Sci.* 19, Proc. 1290, 1960.
9. Smith, S. E., and E. J. Larson. *J. Biol. Chem.* 163: 29, 1946.
10. Supplee, W. C. *Poultry Sci.* 40: 827, 1961.
11. Van Campen, D. R. *J. Nutr.* 88: 125, 1966.
12. Van Reen, R. *Arch. Biochem. Biophys.* 46: 337, 1953.