nearly completed when the sedimentation curve is like that of number 2. The prognosis here is good.

Curve 3 is typical of cases which are in the later stages of resolution. The patient is convalescent. During the rapid absorption of material from the lungs in resolution the sedimentation rate remains quite rapid. Not until the lungs are almost clear does the sedimentation rate improve to the extent shown in Curve 3. Most patients leave the hospital before the sedimentation curve shows as much improvement as that in Curve 3.

Curve 4 shows a typical normal curve which is not seen in cases of severe pneumonia until several weeks after the patient has been discharged and pronounced sound and well as judged by the physical findings and the X-ray. This indicates that the sedimentation test is more sensitive to lung pathology than the X-ray or the physical signs.

In cases of severe pneumonia where the patient does not improve but proceeds to grow worse, the sedimentation curve is more rapid and falls to a lower level than that of Curve 1. So, in curves which are more rapid and fall lower than Curve 1, the outlook is bad.

8631 C

Effects of Thorium, Zirconium, Titanium and Cerium on Enzyme Action.

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Roussy, Oberling and Guerin[1] have reported that thorium dioxide is carcinogenic when administered intraperitoneally to rats. Doerr[2] and Hara[3] report that thorium and some of the associated periodic members agglutinate bacteria, red blood cells, spores, and precipitate proteins. The effects produced upon enzymes by carcinogenic substances, especially arsenites and hydrocarbons, have been rather extensively investigated. Recently Sure *et al.*[4] found

* Contribution No. 70 from the Dept. of Biology and Public Health, Mass. Inst. of Technology, Cambridge.

1 Roussy, G., Oberling, C., and Guerin, M., *Bull. Acad. Med.*, 1934, **112**, 809.

2 Doerr, R., *Kolloid Z.*, 1920, **27**, 277.

3 Hara, S., *Arch. Exp. Path. Pharm.*, 1923, **100**, 217.

4 Sure, B., Kik, M. C., Buchanan, K. S., Thatcher, H. S., and DeGroat, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 658.

marked reduction in the blood serum amylase, blood serum esterase and in the trypsin of tumour bearing albino rats. Pancreatic amylase and blood serum phosphatase were only slightly decreased according to their findings. The present communication reports the results of a series of experiments upon the effects of thorium, zirconium and titanium which are members of Group IV-A of the Periodic System, and cerium, which is a Rare Earth metal but which shows many physiological properties in common with these others, upon amylase, invertase, phosphatase and trypsin activity. The salts employed were thorium nitrate, $Th(NO_3)_4$; cerium nitrate, $Ce(NO_3)_3 \cdot 6\ H_2O$; zirconium nitrate, $Zr(NO_3)_4$; and titanous chloride, $TiCl_3$, which was allowed to oxidize and must be considered as tetravalent titanium.

Amylase activity was determined by estimating the amount of maltose liberated from a one per cent starch solution by a purified bacterial amylase *(B. mesentericus)* (Kindly supplied by the Wallerstein Laboratories, N. Y.) by the iodine titration method of Willstatter-Schudel.[5] A veronal buffer[6] at pH 6.8 was employed. Inorganic phosphate buffers were avoided since the phosphates of the rare metals are very insoluble. Twenty-five cc. of one per cent starch solution, 10 cc. veronal buffer, 2.0 cc. of 10^{-2} M rare metal salt were brought to 60°C. and 1.0 cc. of enzyme solution containing 2.0×10^{-2} mg. solid per cc. was added. The final rare metal salt concentration was 5.26×10^{-4} M. Incubation was at 60°C. The results of an experiment are given in Table I. It can be seen that only thorium exerts any appreciable inhibition of amylolytic activity. From a preliminary experiment without buffer all of the metal salts completely inhibited enzyme activity due to the marked acidity of the salts. Cerium, however, showed slight activation over the control, approximately 30%, as determined both by the time to reach the achromic point and by the amount of maltose liberated from the substrate. The thorium-inactivated amylase could be re-activated by the addition of an excess of 25% KH_2PO_4-

TABLE I.

Influence of Salts of Rare Metals on the Hydrolysis of 1% Starch by Purified Amylase (*B. mesentericus*) at 60° C. Rare Metal Concentration = 5.26×10^{-4} M.

Time (min.)	10	20	30	45	60
		% Inhibition			
Thorium	47.7	39.3	31.5	33.5	45.4
Zirconium	3.8	10.5	6.3	4.4	9.0
Titanium	9.2	14.6	11.6	16.5	23.4

5 Willstatter, R., *Unters. uber Enzyme,* Berlin, 1928, 1, 2.

6 Michaelis, L., *J. Biol. Chem.,* 1930, **87**, 33.

K_2HPO_4 (1:1) which precipitates the metal as the insoluble phosphate. The precipitate is filtered off and the supernatant shows complete re-activation. Similar results were obtained with *taka-diastase* (1:200).

Invertase or saccharase activity was determined both by measuring the amount of reducing sugar produced in the hydrolysis of a sucrose-buffer solution by the method of Sumner and Howell,[7] and by determining the rate of hydrolysis, polarimetrically, of a standard buffered sucrose solution by a purified yeast invertase preparation (Wallerstein). The substrate was 6.5% sucrose-acetate buffer solution at pH 4.5. The enzyme preparation employed in the Sumner-Howell reducing sugar method was a washed suspension of yeast cells (0.5 gm. per cc. in 0.85 NaCl). Five cc. of the substrate, 1.0 cc. of 5.0×10^{-3} M rare metal salt solution and 1.0 cc. of cell suspension were incubated at 25°C. and, periodically, analyses were made for reducing sugar by the dinitro-salicylic acid method of Howell.[8] The results are shown in Table II.

Using 1.0 cc. of 1.0% purified invertase solution in place of the cell suspension the rate of hydrolysis was determined polarimetrically at 25° and the calculated reaction constants are recorded in Table II. The magnitude of the effects produced by the salts of these rare metals can be conveniently estimated by a comparison of the relative rates of hydrolysis as indicated in the table. Again we find that cerium accelerates the enzyme action while thorium, zirconium and titanium produce slight inhibition with titanium showing the most marked effect.

The results of the experiments indicate that action is not due to the agglutination of the cells by the rare metals since the same results were obtained with the soluble enzyme as were obtained with the cell suspension. Moreover, cerium accelerates activity in spite of the fact that it agglutinates the cells.

The effects of the rare metal salts on phosphatase activity were determined by the method described by Bodansky[9] and the inorganic phosphate liberated was determined by the method described by Fiske and Subbarrow.[10] One cc. of fresh, clear horse blood serum was added to 9.0 cc. of buffer-phosphate ester (2.5 gm. sodium-β-glycerophosphate (E. K. Co.) and 2.12 gm. sodium barbital in 450 cc. distilled water) and 1.0 cc. of rare metal salt to give a

[7] Sumner, J. B., and Howell, S. F., *J. Biol. Chem.*, 1935, **108**, 51.
[8] Sumner, J. B., *J. Biol. Chem*, 1925, **65**, 393.
[9] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.
[10] Fiske, C. H., and Subbarrow, Y., *J. Biol. Chem.*, 1925, **66**, 387.

TABLE II.
Influence of Salts of Certain Rare Metals on Production of Reducing Sugars from Sucrose by Yeast Invertase at 25°. Rare Metal Concentration = 7.14 x 10^{-4} M.

Time (min.)	Control	% of reducing sugar produced compared to control			
		Thorium	Titanium	Zirconium	Cerium
5	100	95.3	92.2	102.6	108.1
10	100	102.6	62.5	80.0	121.0
20	100	94.3	61.4	84.4	122.6
30	100	91.3	41.7	85.8	111.1

Effect of Salts of Certain Rare Metals on the Reaction Constant.

Time of hydrolysis (min.)	$K_{control}$ (calculated)	K_{Th}	K_{Ti} (calculated)	K_{Zr}	K_{Ce}
5	.0413	.0393	.0224	.0352	.0435
10	.0333	.0328	.0275	.0314	.0357
15	.0320	.0293	.0270	.0293	.0346
20	.0265	.0244	.0229	.0250	.0295

The reaction constant, K, was calculated with the use of the equation $K = 1/t \log (a/a - x)$; time, *t*, is expressed in minutes; for *a* is used the difference between the observed rotation at 0 time and that of an equilibrium mixture of the hydrolytic products of the sucrose solution; (a — x) is taken as the observed rotation at time *t*, minus the rotation of the equilibrium mixture: $K = 1/t \log (\alpha_0 - \alpha_\infty) - \log (\alpha_t - \alpha_\infty)$

Invertase concentration was 0.143%; the final salt concentration was 0.000714 M. Temperature of hydrolysis, 25° C.

final concentration of rare metal salt of 2.73 x 10^{-3} M, 1.83 x 10^{-3} M, 9.1 x 10^{-4} M, 4.6 x 10^{-4} M and 2.3 x 10^{-4} M at 37°C. Incubation was continued at 37° for 1.0 and for 24 hours. The results tabulated in Table III are expressed as the per cent inhibition as measured against a control in which distilled water replaced the rare metal salt. From the results it is apparent that titanium and zirconium inhibit blood phosphatase very markedly even in very low concentrations. Thorium shows slight inhibition but cerium inhibits only in very high concentrations.

The effects of the rare metal salts on trypsin activity were determined using the method of Willstatter *et al.*[11] Casein solution

TABLE III.
Per cent Inhibition Produced by Various Concentrations of Salts of Certain Rare Metals on Blood Phosphatase after 1 hr. and 24 hr. Incubation at 37° C.

Salt conc.	Thorium		Cerium		Zirconium		Titanium	
	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.
0.00273 M	39.4	28.6	28.6	0	80.6	59.0	90*	86.0
0.00182 M	33.3	25.0	13.0	0	65.0	50.0	90*	81.0
0.00091 M	25.0	22.3	13.0	0	51.1	46.8	66.1	49.2
0.00046 M	23.0	15.3	0	0	48.9	22.0	53.1	48.0
0.00023 M	9.0	11.8	0	0	31.0	20.0	42.0	44.3

*More than 90% inhibition.

11 Willstatter, R., *Z. physiol. Chem.*, 1926, **161**, 191.

(6.5%) was digested with commercial trypsin at pH 8.9 in the presence of the rare metal salts and in an untreated control. Even in concentrations as high as 1.1 x 10^{-3} M no effects could be observed.

Experiments with purified lipase and esterase which will be reported in a subsequent communication indicate that these enzymes are not appreciably affected by the salts studied.

It is questionable whether these results obtained *in vitro* conditions represent the effects that would be observed *in vivo*. The results observed by Sure *et al.*[4] are not paralleled completely since we have found that trypsin, lipase and esterase are unaffected or only slightly affected by these metals, one of which has been reported as carcinogenic. A more justifiable comparison must await the results of the effects of these metals on *in vivo* enzymic activity.

8632 C

Sex-Difference in Susceptibility to Dinitrophenol Intoxication in Anesthetized Cats.*

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In the course of an investigation concerning the interaction of the calorigenic actions of 2-4 dinitrophenol and epinephrine, we have observed a marked sex difference in the susceptibility to dinitrophenol intoxication, as judged by the survival time to a dose which was, under our experimental conditions, almost invariably fatal. There do not appear to have been any reports in the literature concerning sex-differences in the action of dinitrophenol.

Adult cats, previously starved for 24 hours, were anesthetized with pentobarbital (37.5 mg. per kilo intraperitoneally), and attached to a closed circuit metabolism apparatus by means of a tracheal cannula. After a preliminary control period of one-half hour, a quantity of sodium 2-4 dinitrophenolate adequate to yield 10 mg. per kilo of dinitrophenol was injected intramuscularly. In certain experiments, an epinephrine solution was infused intravenously at a rate of 0.001 mg. per kilo per minute during the period

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