to have androgenic activity and to contain a compound giving the reaction for 17-ketosteroids. Its exact chemical structure has not been established due to the small quantity of the substance available for analysis.

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Action of Calcium on Aerobic and Anaerobic Phosphorylation in Malignant and Certain Normal Tissues. (18939)

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In a previous communication(1) measurements of phosphorus uptake by homogenates of liver, brain, and tumor, and by the particulate and supernate fractions obtained by centrifuging such homogenates, were reported. The measurements were made under both aerobic and anaerobic conditions; the results indicated that phosphorylation could be effected by two different systems. In one system, associated chiefly with the particles and more prominent in liver than in tumor homogenates, phosphorus uptake was coupled with aerobic oxidation of succinate or other substrates of the tricarboxylic acid cycle; in the other system, associated chiefly with the supernate and quantitatively more important for the tumor homogenates, phosphorus uptake was coupled with anaerobic, glycolytic breakdown of hexose diphosphate.

Phosphorylation by the aerobic particulate system was, in confirmation of previous workers(2,3), blocked by 10^{-6} to 10^{-5} M 4,6-dinitro-o-cresol (DNC); in contrast, the phosphorylation by the glycolytic mechanism was not impaired by 10^{-4} to 10^{-3} M DNC. The present experiments deal with the sensitivity of these two phosphorylating systems to calcium ion.

Results. The effects of calcium upon phosphorus uptake by homogenates, particles, and supernate were measured with hexose diphosphate and pyruvate as substrate under exactly the same conditions as those described in the previous paper(1). Under these conditions part of the phosphorylation by the homogenate is associated with aerobic oxidation of pyruvate (chiefly by the particles) and part with glycolysis of hexose diphosphate (chiefly by supernate).

Calcium ion reduced the phosphorus uptake by particles from liver from 7.3 to –0.4 micromoles and by particles from brain from 8.5 to 1.5; phosphorus uptake by particles from the tumor, already low in the controls, was not further reduced by 0.0028 N calcium ion (Table I). The effect of calcium on phosphorus uptake by liver and brain particles under these conditions was a specific one, as oxygen consumption and lactate production were not significantly impaired at concentrations up to 0.0028 N Ca^{++}.

The phosphorus uptake by the supernate fraction from liver, brain, or tumor was not inhibited by calcium at concentrations up to 0.0028 N (Table I); there was also no impairment of oxygen consumption or lactate formation by these supernate fractions. The discrepancy between the phosphorus uptake of the tumor homogenate and the sum of the phosphorus uptakes of the washed tumor particles and supernate was found, as in the previous paper, to be due to the fact that when the tumor particles were shaken with a volume of buffered KCl equal to that of the original homogenate and recentrifuged, the wash supernate was found to contain an unexpectedly large amount of the glycolytic phosphorylating mechanism previously found in the original supernate. Since it appeared improbable that such large amounts of this

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TABLE I. Effect of Calcium on Phosphorus Uptake, Lactic Acid Production, and Oxygen Consumption by Homogenates of Rat Tumors, Brains, and Livers, and by the Particulate and Supernatant Fractions Derived Therefrom by Centrifuging at 20,000 g for 30 Min; the Particles Were Washed Once with Buffered KCl, Recentrifuged and Resuspended in Buffered KCl. Incubation time, 40 min at 25°C. Substrate, hexose diphosphate and pyruvate. Gas phase, air. Each experiment is based on material derived from 250 mg original tissue; for other experimental details see (1). All results are expressed in μMols.

Preparation	Conc. of $CaCl_2$, equivalents per l	Normal rat liver			Normal rat brain			Walker tumor 256		
		P uptake	Lactate formed	O_2 uptake	P uptake	Lactate formed	O_2 uptake	P uptake	Lactate formed	O_2 uptake
Homogenate	0	17.6	3.8	10.5	18.7	6.2	8.1	13.2	10.2	2.2
	0	17.7	3.3	10.3	19.3	6.4	8.2	13.5	10.5	2.7
	.00035	17	4.2	11.2	17.2	5.3	8.1	12.5	10.8	2.8
	.0007	16	3.7	13	15.8	6.9	8.5	10.2	11.1	2.4
	.0014	7.9	4	14.4	12	6.2	7.8	9.6	10.7	2.6
	.0028	5.2	4.4	13.6	9.8	6.6	7.8	8.8	10.7	2.7
Particles	0	7.3	1.1	7.3	8.5	3	4.8	2	3.7	1.7
	0	7.3	.7	8.1	8	3.2	4.9	1.9	3.7	1.4
	.00035	7.1	.6	8.9	5.6	3	4.6	2.3	3.4	1.5
	.0007	2.1	.8	9.6	5.6	3	4.7	1.7	4.1	1.4
	.0014	1.5	.6	7.1	2.9	3.4	4.6	2	3.6	1.5
	.0028	−.4	.6	5.7	1.5	2.5	4	1.9	3.3	1.2
Supernate	0	8.1	6.2	2.6	9.8	6.4	2.7	7.3	9.1	2
	0	8.3	5.8	2.4	9.8	6.3	2.6	7.5	8.4	2.2
	.00035	8.7	5	2.7	9.7	6.3	2.6	6.8	8	2
	.0007	8.7	7.5	3.4	9.6	6.8	2.7	7	8.2	2.5
	.0014	8.5	7.8	3.9	9.8	7.1	2.6	7.6	8	2.2
	.0028	8.7	7.8	3.8	9.9	8	2.6	6.8	8	2.2

material could be obtained by merely washing the particulate components, but must be derived from the particles themselves, either by diffusion out of or by partial disintegration of the particles, this matter has been made the subject of a special investigation, the results of which will be reported in a forthcoming publication.

The effects of calcium upon the phosphorus uptake which is coupled with aerobic disposal of α-ketoglutarate were also determined* (Table II). The phosphorus uptake of washed liver particles was blocked completely by about 0.001 N calcium; under these test conditions, in contrast to those obtained with the different test conditions of Table I, there was a substantial inhibition by calcium of oxygen uptake.†

Inhibition of phosphorus uptake by 0.00035 N Ca^{++} was prevented by 0.0014 N citrate (Table II); that by 0.0007 N Ca^{++} was not completely overcome by 0.0028 N citrate.

Repeated attempts to obtain washed particles from a tumor homogenate that would give a phosphorus uptake with α-ketoglutarate as substrate, using the system employed in the experiments with liver particles reported in Table II, have been uniformly unsuccessful.

In contradistinction to calcium, magnesium appeared to exert no effect on the phosphorus uptake and oxygen consumption by the washed liver particles. Magnesium chloride is present in the system employed at a concentration over 10 times that at which calcium completely blocked oxidative phosphorylation. In an experiment in which the magnesium content was varied over a range from 0.0132 M to 0.0016 M the phosphorus uptake and oxygen consumption remained unchanged.

A review of the results reported in this and the preceding paper brings out the fact that while oxidative phosphorylation appeared

* The medium employed in these experiments (Table II) was the same as that employed in those reported in Table I and in the preceding paper with the exception that potassium bicarbonate, nicotinamide, diphosphopyridine nucleotide, and hexose diphosphate were omitted.

† Lehninger(4) has noted that calcium chloride, when employed at a concentration of 0.01 N, inhibited aerobic phosphorylation by washed liver particles, but he does not appear to have reported the effect of the calcium ion on phosphorylation in the lower critical ranges which have been dealt with here.

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TABLE II. Effect of $CaCl_2$ and Sodium Citrate, Singly and in Combination, on Phosphorus and Oxygen Uptakes by Rat Liver Particles Derived from a Rat Liver Homogenate by Centrifuging at 2,000 g for 15 Min; Particles Were Washed Twice with Buffered KCl Solution and Resuspended in Buffered KCl. In each experiment 250 mg wet wt of washed and centrifuged particulate system was employed. Substrate, α-ketoglutarate. Gas phase: air. Incubation time, 40 min at 25°C; results expressed in μMols.

Conc. of $CaCl_2$ equivalents per l	Conc. of sodium citrate equivalents per l	Experiment No. 1		Experiment No. 2	
		P uptake	O_2 uptake	P uptake	O_2 uptake
0	0	16	5.6	17.2	5.7
0	0	15.9	5.2	17.2	4.9
.00018	0	15.9	5		
.00035	0	5.7	2.2	5.8	2.7
.0007	0	1.3	1.9	1.4	1.6
.0014	0	−2.6	.9		
.0028	0	−2.6	1		
0	.00035	16	4.8		
.00035	.00035	4.3	2		
0	.0007	16	4.9		
.00035	.0007	8.5	2.6		
.0007	.0007	1.8	1.2		
.0014	.0007	−5.4	.5		
0	.0014	16	5.3		
.00035	.0014	15.5	4.5	9.4	3.1
.0007	.0014	4.4	.9		
.0014	.0014	−4.4	.8		
0	.0028			17.2	5
.0007	.0028			3.4	2.1

to be always dependent on the presence of particles and the water-soluble phosphorylating mechanism that appears in the supernate is invariably anaerobic in type, there is some evidence in the case of tumor and brain particles but not liver particles that an anaerobic mechanism resistant to DNC, calcium chloride, and anaerobiosis is either associated with or actually contained within some particulate component. This problem will be made the subject of a future investigation.

Summary. Phosphorus uptake and oxygen consumption by particles from rat liver, with α-ketoglutarate as substrate, are almost completely inhibited by 0.0007 N Ca^{++} and completely inhibited by 0.0014 N Ca^{++}; this effect may be a specific one upon phosphorus uptake or transfer, as the oxygen uptake with pyruvate and hexose diphosphate as substrate is not markedly impaired at the same Ca^{++} concentrations. Citrate used in concentrations equivalent to the calcium had no protective effect against the Ca^{++} inhibition, but larger ratios of citrate to calcium afforded partial or complete protection. Attempts to obtain oxidative phosphorylation with washed particles obtained from Walker 256 rat tumor homogenates, using α-ketoglutarate as substrate, were unsuccessful. Wide variations in concentration of magnesium appeared to exert no effect on the phosphorus uptake and oxygen consumption of washed rat liver particles.

When homogenates, particles, and supernates of rat liver, rat brain, and the Walker rat tumor 256 were subjected to the action of calcium with hexose diphosphate and pyruvate as substrate, the maximal inhibiting effect on the phosphorus uptake of homogenates and particles was noted with liver, an intermediate effect was obtained with brain, and a minimal effect was found with tumor. In all cases the phosphorus uptake, lactate production, and oxygen uptake of supernates were unaffected by calcium chloride at concentrations up to 0.0028 N.

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supply of pathologically controlled, Walker 256 rat tumors at suitable stages of development.

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The Effect of Unrelated Vaccines on the Localization of Paralysis in Mouse Encephalomyelitis. (18940)

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Recent studies of poliomyelitis suggest that the inoculation of unrelated prophylactic and therapeutic agents increases the risk of paralysis in the inoculated limb(1-4). The importance of the observation to immunization programs and its implications regarding the pathogenesis of poliomyelitis prompted us to seek an experimental counterpart so that the phenomenon might be studied in the laboratory. The results of mouse tests in which the Lansing strain of poliomyelitis virus was used were irregular but suggested that the injection of vaccine may hasten the onset of paralysis, as has recently been reported(5,6). It was thought that mouse encephalomyelitis might prove more suitable since it is a natural disease of mice and is in so many respects similar to poliomyelitis. The present report describes experiments in which the introduction of vaccines apparently did increase the frequency of paralysis in the inoculated extremity following the intracerebral injection of mouse encephalomyelitis virus.

Methods. The mice used in the present experiments were of the Albany Swiss and Standard strains. Groups of 20 to 60 animals, 3 to 5 weeks old, were assembled by stratified random sampling.

The prophylactic agents tested, fluid-medium pertussis vaccine(7), precipitated diphtheria-tetanus toxoid, precipitated diphtheria-tetanus toxoid with pertussis vaccine, and typhoid-paratyphoid vaccine, were prepared in this laboratory(8). The bacterial count of the pertussis vaccine and of the triple combined product was 20,000 million per ml; that of the typhoid-paratyphoid vaccine 1,500 million per ml. The vaccines and other test substances were injected subcutaneously in the left front leg in 2 doses of 0.1 ml amounts a week apart at varying intervals in relation to the virus inoculation. A front leg was chosen for the site of vaccine injection because the virus strain employed induces paralysis in the front legs less frequently than in the hind legs(9).

A TO strain of mouse encephalomyelitis (Theiler) virus, No. 4727, was used throughout. Stock 5% mouse brain suspensions were stored until use in a dry-ice cabinet. The virus was injected into the left cerebral hemisphere in doses of 0.03 ml of 10^{-2} or 10^{-5} dilutions. Control groups received virus or vaccine only or saline in place of vaccine followed by virus. The mice were observed daily for at least 21 days following the inoculation of the virus. Mice were destroyed as soon as paralysis appeared.

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