No. 2 and the mouse Ehrlich cells.

Discussion. In presenting these results, we wish to call attention to anti-mammalian cellular activity of extracts of the *Lamptero-myces japonica* (L.J.) fungus. One partially purified fraction appears to be active against human cancer cells in tissue culture in microgram quantities and is not inactivated by serum. It acts on the Ehrlich tumor cell *in vivo* in mice in subtoxic doses providing it comes in contact with the cells. Whether or not further purification and characterization of the active principle in fraction No. 4 of the L.J. extract will lead to a more active fraction remains to be seen. The senior author has made some progress in isolating and characterizing this substance which appears to contain little if any nitrogen. Whether or not such material injected intravenously will have an effect on an established tumor of animals or man is questionable unless procedures are developed for selectively increasing venule or capillary permeability to macromolecular substances in the tumor bearing area. This problem is being investigated.

The chemical differences in the surfaces of cancer cells and normal cells appear to be real but small, as indicated by current immunological studies. Furthermore, there is no reason to believe that all tumor cells have a common chemical surface component different from normal cells. Therefore, in searching for specific chemotherapeutic anticancer agents, one might well consider macromolecular substances which, by nature of their size, are most likely to have a characteristic surface configuration and thus have a specific action.

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Radiomanganese Studies in Pyridoxine Deficient Rats. (27890)

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Our earlier publication(1) and that of Hartsook and Hershberger(2) have shown that deficiency of pyridoxine in rats results in a disturbance of cellular equilibrium of sodium and potassium contents in tissues. Using isotopic technics, Hsu(3) has recently reported that pyridoxine deficiency decreases I^{131} uptake by the thyroid glands in rats. Further observations(4) in rabbits have indicated that administration of deoxypyridoxine, an antagonist of pyridoxine decreased the concentration of serum magnesium, and reduced uptake of Mg^{28} tagged chloride in kidneys, lung and bone. All the above findings demonstrated that pyridoxine plays an important role in maintaining the electrolyte balance. The present report indicates that deficiency of pyridoxine also disturbs the normal tissue distribution and rate of excretion of another indispensable trace metal, manganese, in rats.

Materials and methods. Pyridoxine deficiency was produced in young adult rats of both sexes of the McCollum strain by feeding a high protein diet* deficient in pyridox-

* Basal diet: Vitamin free casein 27, sucrose 59, corn oil 10, Salt mixture 4 and vitamin supplements: See Hsu and Chow, *Arch. Biochem. and Biophys.,* 1957, v72, 322.

TABLE I. Effect of Pyridoxine Deficiency on Growth, Plasma, GOT and Erythrocytes GSH in Female Rats.

Treatment	Initial body wt, g	Final body wt, g	Plasma GOT, units/ml	Erythrocytes GSH, μmole/100 ml
Pyridoxine-deficient	163 ± 8.3*(5)	207 ± 7.8‡	43 ± 5‡	261.6 ± 20.4†
Pyridoxine-treated	155 ± 5.2 (5)	276 ± 9.8	152 ± 13	178.9 ± 12.6

* Mean value and standard error of mean. Figures in parentheses indicate No. of rats used.
† Statistically significant difference from pyridoxine treated rats, $P < .05$.
‡ Statistically significant difference from pyridoxine treated rats, $P < .01$.

ine. The control animals were kept on the same basal diet supplemented with pyridoxine HCl(20 mg/kg). Deoxypyridoxine HCl (0.75 mg/0.5 ml) in isotonic saline solution was given intraperitoneally to the female rats 3 times weekly for the last 2 weeks in order to intensify the deficiency of Vit. B_6. At the end of the 5- to 6-month feeding period each rat received a single intravenous injection of 5 to 10 μc of carrier-free Mn^{54} tagged chloride in 1.0 ml of distilled water by way of an external great saphenous vein. The animals were transferred to individual metabolism cages for separate collection of urine and feces, and were killed by cardiac puncture at intervals of 18, 48 and 120 hours following injection. The heparinized cardiac blood was centrifuged to separate the plasma. The various tissues were immediately removed, cleaned with a normal saline solution, and liquefied by adding an aqueous solution of 30% potassium hydroxide. Urine was concentrated over a steam bath whereas the feces were softened with water and solubilized with concentrated sulfuric acid. The Mn^{54} radioactivity in aliquots of the tissues and excreta was measured by means of gamma scintillation spectrometry. The results are expressed as per cent of injected dose.

In one experiment, plasma was used for estimation of glutamic-oxalacetic transaminase (GOT) activity according to the method of Reitman and Frankel(5). One ml of whole blood after completed hemolysis with distilled water was deproteinized by mixing with 2.0 ml of 5% metaphosphoric acid. The clear supernatant was applied for the determination of reduced glutathione (GSH) using alloxan '305' method(6).

Results and discussion. Pyridoxine deficiency in the rats was indicated by 3 criteria: retardation of growth rate, decrease of plasma transaminase activity, and elevation of glutathione concentration in erythrocytes as shown in Table I. The present findings confirm that pyridoxine deficiency induced in rats by a high protein diet or a high fat diet is accompanied by a disturbance of glutathione metabolism(7).

The data in Table II demonstrate that the Mn^{54} is most rapidly eliminated by way of the gastrointestinal tract. About 40% of the injected radioactivity was found in the gastrointestinal tract of pyridoxine deficient rats in 18 hours compared to approximately 30% for the pyridoxine treated animals. The difference is statistically significant with $P < 0.05$. Liver contained one quarter of the injected dose of radioactivity, but much less

TABLE II. Effect of Pyridoxine Deficiency on Uptake of Mn^{54} in Various Tissue of Male Rats (18 Hr Following i.v. Inj of 10 μc $Mn^{54}Cl_2$).

Treatment	Radioactivity (% of i.v. dose)						
	Brain	Liver	Kidney	Spleen	Heart	G.I. tract	Testicles
Pyridoxine-deficient	.2235 ±.026* (5)	28.33 ± 1.11	6.19 ±.29	.611 ±.047	.603 ±.05	39.97 ± 1.82†	1.38 ±.08†
Pyridoxine-treated	.2670 ±.025 (5)	27.68 ± 2.18	7.30 ±.64	.712 ±.027	.734 ±.06	29.02 ± 2.85	1.12 ±.04

* Mean values and standard error of mean. Figures within parentheses indicate No. of rats used.
† Statistically significant difference from pyridoxine treated rats, $P < .05$.

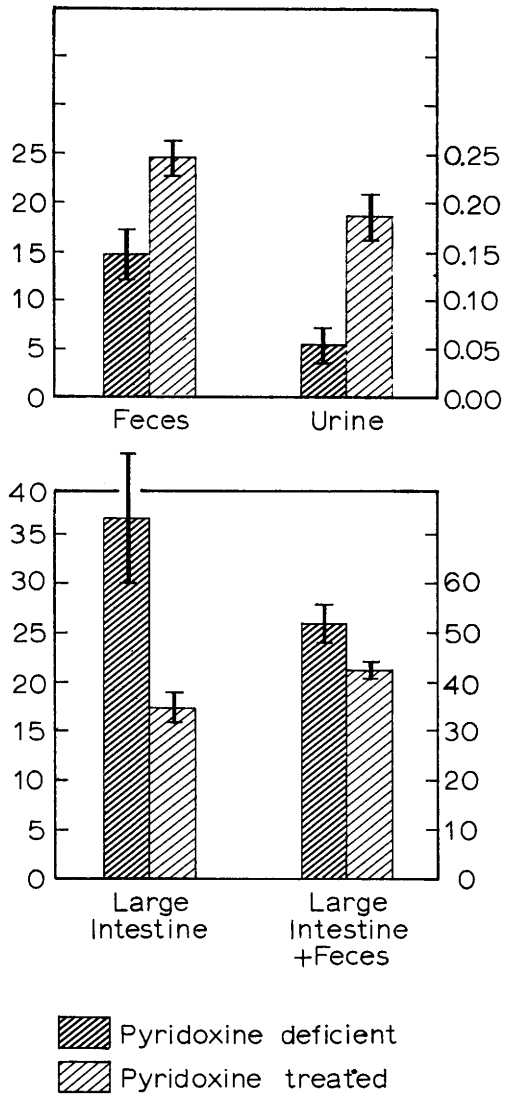

FIG. 1. Excretion of Mn^{54} in urine, feces, and large intestine. (Expressed as percentage of inj dose.)

was recovered from kidneys, brain, spleen, and heart. Radiomanganese concentration in testicles was higher in the deficient animals.

Effects of pyridoxine deficiency on excretion of Mn^{54} in feces and urine 48 hours after i.v. injection are shown in Fig. 1. Average radioactivity in feces from pyridoxine deficient rats was about 60% of the value found in pyridoxine treated animals. However, the combined radioactivity in the feces and the large intestine was significantly higher in deficient rats than in pyridoxine treated rats. On the other hand, the amount of the isotope eliminated in urine of pyridoxine deficient rats was considerably reduced.

Table III illustrates effects of pyridoxine deficiency on distribution of Mn^{54} activity 48 and 120 hours following its administration. At both intervals, uptake of Mn^{54} by brain tissue of pyridoxine deficient rats was significantly higher than those of their respective controls. These findings may relate to the well known influence of Vit. B_6 on brain enzyme activity. In contrast to the higher brain concentration of Mn^{54}, pyridoxine deficient rats retained less radioactivity in their livers compared to the control rats. Differences in hepatic uptake between the groups were greater in the rats sacrificed 120 hours after the Mn^{54} injection than in those killed after 48 hours. Uptake of radioactivity by heart tissue was lessened in the depleted animals.

Greenberg and Campbell(8) have pointed out that the major route of excretion of oral or injected manganese is by the way of bile into gut, and is recovered in the feces. That pyridoxine deficient rats excreted more radioactivity in the feces and the large intestine after injection of radiomanganese suggests that the rate of excretion of the metal is somewhat increased in the presence of pyridoxine deficiency. The results also show that deficiency of pyridoxine increases the concentration of Mn^{54} in the brain, and decreases the amount of radioactivity in the liver, but has little or no effect on its distribution in other tissues. These findings reveal the possibility of intracellular damage induced in the brain and liver tissues by the restriction of pyridoxine. In view of the primary function of manganese as an enzymatic catalyst in the Krebs cycle, it is possible that abnormal metabolism of this trace metal might subsequently alter certain metabolic pathways in carbohydrate.

Summary. Tissue distribution and excretion of radioactivity following intravenous injection of $Mn^{54}Cl_2$ at 3 different time intervals were studied in rats deficient in pyridoxine and in control animals. The findings

TABLE III. Effect of Pyridoxine Deficiency on Intravenous Mn^{54} Tissue Distribution.

Treatment	48 hr post inj interval		120 hr post inj interval	
	Pyridoxine deficient (6)	Pyridoxine treated (5)	Pyridoxine deficient (5)	Pyridoxine treated (4)
Brain	.312 ± .06*†	.283 ± .017	.367 ± .008†	.317 ± .022
Heart	.382 ± .03†	.477 ± .04	.244 ± .01	.289 ± .02
Liver	22.35 ± .3 †	24.74 ± .2	13.36 ±1.34 †	17.84 ± .72
Kidney	4.13 ± .24	3.54 ± .19	2.56 ± .07	2.52 ± .29
Spleen	.519 ± .04	.698 ± .02	.263 ± .02	.330 ± .03
Lung	.565 ± .02	.513 ± .07	.398 ± .03	.412 ± .05
Pancreas	—	—	2.46 ± .51	2.22 ± .45
G.I. tract	—	—	28.28 ±2.27 ‡	13.30 ±2.26

* Mean values and standard error of mean. Figures in parentheses indicate No. of rats used.
† Statistically significant difference from pyridoxine treated group, $P < .05$.
‡ Statistically significant difference from pyridoxine treated group, $P < .01$.

indicate that deficiency of pyridoxine is associated with increased uptake of the injected dose by brain and testicles, and with decreased uptake by liver and heart. No differences in Mn^{54} concentrations were observed in other tissues examined. The tracer content in the gastrointestinal tract and the feces of the pyridoxine deficient rats is significantly higher than in those of their respective controls. However, urinary excretion of radioactive manganese is reduced in pyridoxine deficient groups. These results suggest a role of pyridoxine in the regulation of manganese metabolism.

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Adipokinetic Effect of Intravenous Cortisol in Human Subjects.* (27891)

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The role of the adrenal cortex in regulation of lipid metabolism and transport in man is still uncertain(1). Elevation of serum lipids and O-lipoprotein has been observed in patients with Cushing's syndrome and in patients given cortisone therapy(2). No data are available relating adrenal corticosteroid administration to levels of serum fatty acids nor to lipid mobilization *in vivo* from adipose tissue in man(3). The present study was undertaken to investigate the acute effects of cortisol administration on levels of serum free fatty acids in healthy human subjects.

Experimental procedure. Materials and methods. Eighteen healthy volunteers were the subjects of the study. Eleven women,

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