compound, liver pyridine nucleotide concentration was significantly lower in the Vit. B_6-deficient rats than in the control rats. Liver pyridine nucleotide values were also significantly lower in the deficient rats at 1 and 4 hours after injection of nicotinic acid but not after injection of nicotinamide.

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Chemotherapy of Experimental *Leishmania enriettii* Infection. (27231)

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L. enriettii, first described by Muniz and Medina, produces a muco-cutaneous leishmaniasis of guinea pig which is not transferable to other species(1). Muniz(1), Adler (2), Paraense(3) and Torres *et al.*(4) have described the biological and pathological characteristics of the organism.

Extending our studies(5) on treatment of the cutaneous leishmaniasis with *L. brasiliensis* responsible for the clinical condition known as *Leishmaniasis tegumentaria diffusa* (6), we have examined the effect of drug treatment on *L. enriettii* infection produced experimentally in the laboratory.

Materials and methods. The strain forthcoming from Dr. Julio Muniz, Instituto Oswaldo Cruz, was obtained by us in Jan. 1960 from Professor Enrique Tejera. For experimental infection, we injected guinea pigs intracutaneously with a suspension of infected tissue into the nasal region. In 115 guinea pigs, the lesions developed within 20 and 40 days after inoculation, except in 5 animals in which symptoms appeared after 10 days and in one, with delayed reaction (90 days). The foreleg also appeared a sensitive site for inoculation; 15 out of 17 pigs so infected developed wide lesions within 21 days, the remaining 2, within 52 days. Daily subcutaneous treatment, 6 times weekly, was given during the period indicated to the animals showing well developed lesions, except for Sodium-stibogluconate (Pentostam), which was injected intraperitoneally, twice daily. The animals were observed grossly and microscopically twice a week.

Results. The effect of therapy on regression of lesion and number of leishmania is contained in Table I. Fig. 1 and 2 illustrate the effects of drug action. It appears that Fuadin, given up to 18 to 21 times in doses of 300 mg/kg, remained ineffective, as did tartar emetic given 35-51 times in 15 mg/kg doses. No effect followed administration of maximal hydroxy-stilbamidine doses which could be administered repeatedly (25 mg/kg). Pentostam given 58 times in 25-50 mg/kg (i.p.) doses did not interfere with the progress of the infection. The only drug tested which showed a prompt effect in tol-

erated doses was Antimoniate of N-methyl-glucamine (Glucantime). After 6 to 15 treatments with 0.5-1.0 g/kg the lesions decreased and no leishmania were found, while after 35-45 treatments the lesion disappeared completely. Out of 8 guinea pigs treated with glucantime, observed for over one year, only 2 relapsed, within the first month after last treatment.

Conclusions. There is a parallelism between the chemotherapeutic results observed by us(5) in the cutaneous *L. brasiliensis* infection of mice, and those obtained in the cutaneous guinea pig (*L. enriettii*) leishmaniasis.

According to results so far obtained, cutaneous infection of the guinea pig with *L. enriettii* has the drug sensitivity of cutane-

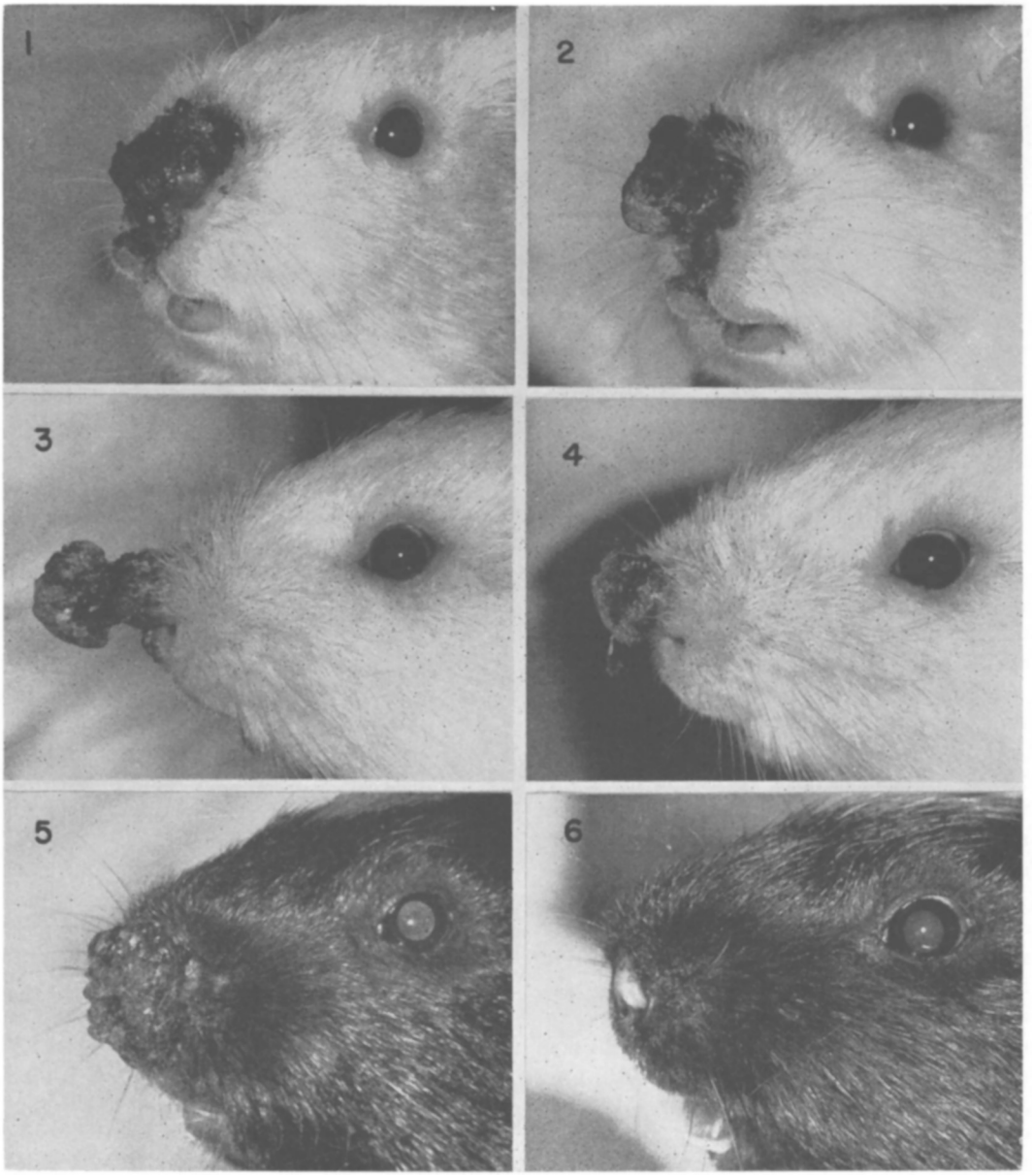

FIG. 1. 1. Guinea pig 220 6 wk after inoculation, before treatment. 2. After 26 Fuadin treatments, 100 mg/kg. 3. After 32 treatments. 4. After 45 treatments. 5. Guinea pig 234 before treatment. 6. Same animal cleared after 32 × 1 g/kg Glucantime.

ous *L. brasiliensis* infection, which is not affected by Pentostam, tartar emetic and hydroxy-stilbamidine. Glucantime treatment gave prompt therapeutic results. It is likely that the higher effect of Glucantime depends on its great tolerance and on the large amounts of pentavalent antimonials which can be given in this form. The *L. enriettii* infection, not contagious for man, represents under these conditions a useful method of searching for new therapeutic agents effective against the human form of cutaneous leishmaniasis. Both these cutaneous infections have a different drug sensitivity than *L. donovani;* whether this difference depends

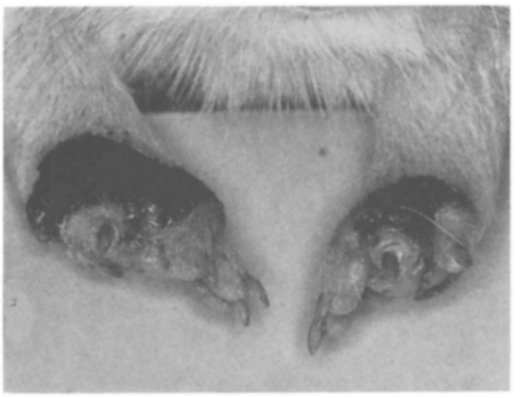

FIG. 2. Guinea pig 13 infected subcut. in foreleg, after treatment with 10 × 25 + 48 × 50 mg/kg Pentostam.

TABLE I. Effect of Chemotherapy on *L. enriettii* Infection of Guinea Pigs.

Animal No.	Drug	Dose, mg/kg s.c.	Initial lesion: Diam., cm	Initial lesion: No. leishm.	Effect after ... treatm.: No. treatm.	Effect after ... treatm.: Diam., cm	Effect after ... treatm.: No. leishm.	Final effect: No. treatm.	Final effect: Diam., cm	Final effect: No. leishm.
195	Fuadin	300	1.0	5/F	24	.8	±	33	0	0/S
194	"	"	2.0	20/F	3	2.0	40/F	9	3.0	Died
120	"	"	1.2	20/F	18	1.0	10/F	21	1.5	2/F
177	"	"	1.4	50/F	18	1.2	5/F	21	.6	20/F
182	"	150	1.4	15/F	6	1.3	30/F	12	1.4	10/F
183	"	"	1.2	25/F	18	1.5	150/F	21	1.4	200/F
219	"	100	2.0	100/F	14	1.2	50/F	28	1.0	50/F
220	"	"	2.0	150/F	32	1.0	10/F			
					45	1.3	20/F	51	1.0	1/F
221	"	50	1.5	150/F	14	1.2	100/F	29	.6	20/F
222	"	"	1.0	150/F	40	.3	10/F	51	.2	1/F
2	Pentostam	48 × 50*	2.0	20/F	20	2.0	500/F	58	2.5	75/F
13	"	"	1.0	30/F	33	1.5	500/F	58	2.5	20/F
114	T. emetic	15	.5	100/F	23	.6	2/F	35	.6	2/F
117	"	"	1.5	500/F	23	1.0	50/F	35	1.2	3/10F
236	"	"	1.5	50/F	14	2.0	75/F	51	.9	5/F
237	"	"	1.5	30/F	32	.8	10/F			
					45	.2	3/S	51	0	0/S
115	Hydroxystilb.	25	1.4	500/F	8	1.4	50/F	12	1.4	+
116	"	"	1.4	10/F	8	1.4	20/F	12	1.4	1/F
227	"	15	2.0	50/F	4	1.2	15/F	10	1.0	100/F
231	"	"	2.0	50/F	4	2.0	30/F	9	2.0	150/F
232	"	7.5	1.8	200/F	9	2.0	100/F	24	2.0	100/F
235	"	"	1.8	10/F	9	2.0	40/F	19	1.4	500/F
185	Glucantime	500	1.0	30/F	15	.8	0/S	45	0	0/S
190	"	"	.8	5/F	6	.7	0/S	45	0	0/S
191	"	1000	1.5	75/F	9	1.2	1/10F	45	0	0/S
193	"	"	1.5	10/F	6	1.3	0/S	45	0	0/S
112	"	"	1.2	50/F	15	.1	0/S			
					23	0	0/S	35	0	0/S[R]
113	"	"	1.6	10/F	15	1.5	0/S			
					23	.3	0/S	35	0	0/S[R]
226	"	"	2.0	50/F	14	.8	0/S			
					32	0	0/S	51	0	0/S
234	"	"	2.5	100/F	14	.8	0/S			
					26	0	0/S	51	0	0/S

[R] = relapsed F = field (×800) S = microscopic slide * i.p.

on the specific drug sensitivity of the infective organisms, or on particular conditions connected with the cutaneous localization of the infections, remains to be determined. In this respect, it is known that post kala-azar dermal leishmaniasis is insensitive to stilbamidine type drugs, which are highly effective in the corresponding systemic infection.

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Comparative Availability of Phytin and Inorganic Phosphorus to Rumen Microorganisms, *in vitro.**† (27232)

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Numerous studies have shown that when dietary calcium is increased availability of phytin phosphorus is decreased for both man and laboratory animals(1-3). and to a lesser extent for cattle(4,5). Marston(6) has reviewed the early work, and Duncan(7) has discussed and summarized recent studies on the role of phosphorus and calcium in ruminant nutrition. Both *in vitro* and *in vivo* experiments have verified the availability of phytin phosphorus to ruminant animals under natural conditions(4-10), indicating the enzyme phytase, produced by rumen microorganisms, can hydrolyze ingested phytates releasing the available phosphorus for absorption. However, excess calcium ions inhibit phytase(11); and the observed rachitogenic effects of cereal diets have been accredited to the unavailability of phosphorus(12). The subsequent variations in available dietary phosphorus have contributed to existing differences of opinions on the relative effects of amount and ratio of calcium to phosphorus (12,13) upon utilization.

The paucity of information concerning individual contributions of phosphorus and calcium levels and ratios to phytin phosphorus availability in the ruminant animal, and the need for data on the contribution of the rumen to utilization of these phosphorus sources, prompted this study.

Methods and materials. A modification of the artificial rumen technic, washed cell suspension, described by Anderson *et al.*(14) was employed. In each series 1200 ml of rumen liquor were taken from a fistulated cow maintained on a grass hay ration. The microorganisms were washed twice and suspended in 600 ml of basal media, complete with the exception of phosphorus. Five g of purified cellulose and 9 ml of a 3% feather meal hydrolyzate were added and a 24-hour preliminary fermentation period employed to deplete the washed cells of any residual phosphorus. Feather meal hydrolyzate served as a stimulant to increase bacterial activity and, subsequently, achieve maximum phosphorus depletion. The microbial suspension was then made up to 1200 ml with the phosphorus deficient media, and 5 g of purified cellulose were added. Twenty ml of this bacterial suspension were pipetted into sets of 3 individual pyrex digestion tubes containing 0.3 ml feather meal hydrolyzate and 2.068 mg phosphorus as phytin ($Ca_5Mg(C_6H_{12}O_{24}P_6 \cdot 3H_2O)_2$) or as a combination of Na_2HPO_4

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