A NOTE ON THE RESORPTION AND EXCRETION OF RIBOFLAVINE FROM ALUMINIUM MONOSTEARATE SUSPENSIONS IN THE RAT

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Nutritional studies in this country have shown that riboflavine deficiency is very frequent[1], and that its incidence is much greater than that of other vitamin deficiencies[2]. The clinical manifestations of this deficiency, such as glossitis, cheilosis and corneal vascularisation, are particularly common during pregnancy. Thus, reduced excretion of riboflavine (averaging 95μg./l. of urine instead of 360 μg./l. as in normal pregnant women) was found in 21 per cent. of 900 pregnant women[3]. Riboflavine deficiency is also associated with prematurity of the fœtus, a high incidence of antenatal death and post partum agalactia or hypogalactia[4]. Surprisingly enough, these clinical manifestations appeared not only in the poorer classes in which riboflavine intake was inadequate, but in well-nourished subjects as well. The latter finding is principally due to inadequate resorption of this vitamin. The frequent occurrence of intestinal and hepatic disorders such as amœbic dysentery, gastric achlorhydria and hypochlorhydria, and various types of chronic hepatitis in undoubtedly the most important etiologic factor in the production of deficiency symptoms in these subjects[5].

Riboflavine therapy generally produces striking improvement in cases in which the deficiency is due to insufficient intake. On the other hand, poor results usually follow oral administration in subjects suffering from deficiency due to inadequate resorption. In these patients even parenterally administered riboflavine is eliminated from the body within a few days[6].

In order to maintain a constant level of riboflavine in the tissues, we have attempted the implantation of pellets so as to afford continuous and adequate resorption of the vitamin. This method of supply seems to us particularly advantageous in patients who cannot be trusted to take medication regularly. For this purpose we prepared pellets containing 50 mg. of riboflavine fused with 50 mg. of cholesterol[7] which on implantation maintained a high level in man and animals for up to $1\frac{1}{2}$ months.

Recently we have simplified the treatment by developing a single injection technique. Two aspects of this type of therapy, the rate of excretion and the rate of recovery from the site of injection of a suspension of riboflavine aluminium monostearate, are reported in this paper.

Technique

Riboflavine suspensions were prepared with 2 per cent. aluminium monostearate*. They are stable when protected from light, easily

* We are indebted to Dr. G. Friedlaender of "Teva" Middle East Pharmaceutical & Chemical Works, Ltd., Jerusalem, Israel, for the preparation of the suspensions and solutions used.

tolerated, contain up to 50 mg./ml. and cause no local reaction on injection. Their effect was compared with that of buffered riboflavine solutions prepared by the same manufacturer.

Twelve male and 12 female rats (Hebrew University strain) weighing 150 ± 10 g. were used. They received standard Purina diet which gave a daily urinary excretion of 10–20 μg. of riboflavine per rat. The rats were placed in groups of 2–4 in metabolism cages with unlimited intake of food and water. The urine was collected once a day in 1 ml. glacial acetic acid; and every 24 hours the cages were rinsed with 100 ml. of tap water which was added to the urine. After a one week period of adaptation, the urine was assayed daily for the vitamins. The rats were then given a single injection of suspension (4 male and 4 female rats to each dose level of 20, 25 and 50 mg.) and the excretion of the vitamin in the urine of the 12 male rats was determined daily by the fluorometric method[8]. The urine was assayed at dilutions containing 0·1–0·2 μg./ml. The excretion was expressed in terms of μg. of riboflavine excreted per rat per day.

TABLE I

AVERAGE URINARY EXCRETION OF RIBOFLAVINE IN RATS INJECTED SUBCUTANEOUSLY WITH SUSPENSIONS OF RIBOFLAVINE ALUMINIUM MONOSTEARATE AND RECOVERY OF RIBOFLAVINE FROM THE SITE OF INJECTION

Days after injection	Total urinary excretion* versus recovery† from site of injection	Riboflavine suspension injected		
		20 mg.	25 mg.	50 mg.
		mg.	mg.	mg.
1–12	Excretion	10·4	14·2	17·8
	Recovery	8·6		28·4
13–24	Excretion	7·5	7·2	10·5
	Recovery	1·2	1·5	9·8
25–36	Excretion	1·1		3·1
	Recovery	0·9	0·6	2·6
37–48	Excretion	0·5		2·1
	Recovery	0·1	0·1	1·4

* Average value of excretion derived from 4 rats.
† Single value of recovery derived from 1 rat.

An additional group of 4 male rats received an injection of 20 mg. of a riboflavine solution and served for comparison with the 3 groups receiving the suspension.

The 12 female rats served mainly for the study of the resorption of the vitamin from the lumps which were removed from the site of injection. Occasionally, their urines were also checked for riboflavine excretion. There were no significant deviations from the excretion found in the male rats. The female animals were killed at intervals of 12 days. The organised riboflavine residues at the site of injection were removed, minced and extracted with 0·1N hydrochloric acid according to the method of the Association of Vitamin Chemists[8].

RESULTS

Riboflavine suspensions injected subcutaneously at dosage levels of 20, 25 and 50 mg. per rat gave average daily urinary excretion as shown in Figure 1. One injection of 20 mg. was enough to maintain an elevated excretion of the vitamin for 52 days. After injection of 50 mg. excretion was increased for 105 days.

For comparison, 4 male rats were injected subcutaneously with 20 mg.

of a riboflavine solution. Average excretion in their urine was increased only during the first 4 days. Seventy-five per cent. of the injected riboflavine was excreted in the urine during the first day and was accompanied by diuresis of 25 ml. as compared with 2·5–5 ml. on a control day. Larger doses of the solution proved to be toxic. The toxicity was not

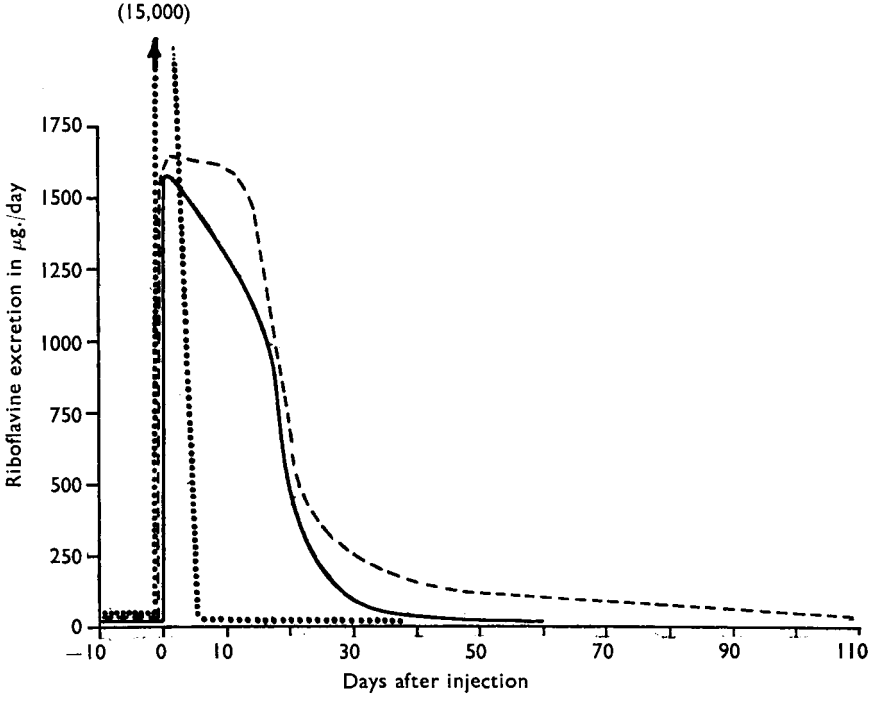

FIG. 1. The excretion of riboflavine in μg./day after injection of:—
20 mg. in suspension (——)
50 mg. ,, ,, (— —)
20 mg. in solution (· · · · ·)

due to the solvent, but rather to the high doses of the riboflavine itself. This question is under further study.

A comparison of the residual riboflavine content in the lumps removed from the site of injection is made with the total excretion in Table I. Riboflavine in the urine is derived from that injected and that contained in the diet. The amount recovered from the urine and the site of injection roughly equalled the amount injected and ingested.

DISCUSSION

The injection of suspensions described here is superior to the implantation method described by us earlier[7]. Whereas 50 mg. implanted provided riboflavine for 45 days only, the same quantity injected as an aluminium monostearate suspension lasted up to 105 days.

The results obtained with the suspension method are quite satisfactory.

The very slow resorption of the vitamin is not only due to slow liberation from the suspension but also to the poor solubility of the vitamin in body fluids, its solubility in an aqueous medium is only 1:10,000.

There exist at present 3 methods of injecting crystal suspensions:

(1) *Crystal formation at the site of injection*: The drug is injected as an aqueous solution with 2 per cent. urethane, the latter is removed by the body fluids, leaving a precipitate at the site of injection which is resorbed within 14–21 days[9].

(2) *Crystal formation in the syringe*: The drug is dissolved in an organic medium and when mixed with normal saline in the syringe, is precipitated as a fine crystalline suspension which becomes coarse after injection in the tissues and is resorbed within 14–21 days[10].

(3) *Crystal formation in the ampoule*: The drug is suspended with the aid of a suitable suspending agent, such as pectin, sodium oleate, magnesium stearate or, as described above, aluminium monostearate before packaging in ampoules.

It seems that the last method using 2 per cent. aluminium monostearate is the most promising. We have therefore begun an investigation on its use in man in collaboration with Drs. Bromberg and Brzezinski. Furthermore the application of this method to steroid hormone therapy will be studied.

Summary

1. The use of riboflavine suspensions with 2 per cent. aluminium monostearate for depot therapy has been studied in rats. A depot of 50 mg. takes up to 105 days to be resorbed and smaller quantities accordingly shorter periods (52 days for 20 mg.). This method of administration is superior to the implantation of pellets previously described by us.

2. The depots formed produced no local reactions and allowed slow and continuous resorption of the vitamin from the site of injection.

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