nen gibt (Figur 3). Eine ganz analoge Charge-Transfer-Bande lässt sich auch durch eine Wechselwirkung von 5,6,7,8-Tetrahydrofolsäure mit Chloranil beobachten. Im Falle der N-Phthaloyl-DL-glutaminsäure würde die Phthalimidstruktur als Akzeptor anzunehmen sein.

Diese Befunde lassen es möglich erscheinen, dass die teratogene Wirkung des Thalidomid auf eine Wechselwirkung der als Metabolit auftretenden N-Phthaloylglutaminsäure (bzw. ihrer Amide) mit Folsäure oder Tetrahydrofolsäure zurückzuführen ist.

Experimentelle Angaben. 1. Solubilisationsversuche. Ein Überschuss von Folsäure (40 mg; für biochemische Zwecke, Merck) wurde mit 0; 3; 5; 6,66; 8 und $10 \times 10^{-2} M$ Lösungen von N-Phthaloyl-DL-glutaminsäure in getrocknetem Dioxan (Merck) 3 h bei 20 °C ($\pm$ 0,1°) geschüttelt. Die ersten 5 ml des Filtrats wurden verworfen. In 10 ml des Filtrats wurde in einem 25-ml-Kölbchen die Folsäuremenge nach Spaltung, Diazotierung und Kupplung der entstandenen N-(4-Aminobenzoyl)-glutaminsäure photometrisch bei 550 nm bestimmt[14].

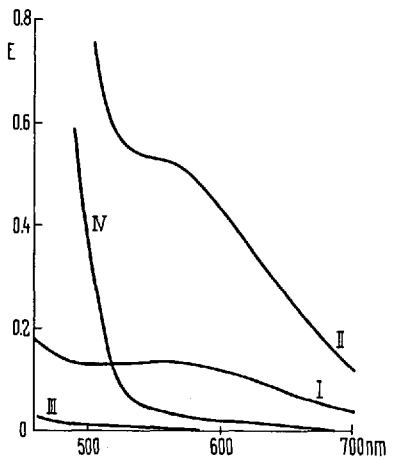

Fig. 3. Charge-Transfer-Banden der Komplexe Folsäure-Chloranil (I) und Tetrahydrofolsäure-Chloranil (II). Kurve III: Extinktion der Folsäure. Kurve IV: Extinktion der 5,6,7,8-Tetrahydrofolsäure. Konzentration an Folsäure bzw. Tetrahydrofolsäure in allen Fällen $5{,}0 \times 10^{-3} M$. Konzentration an Chloranil $5{,}0 \times 10^{-2} M$. Lösungsmittel: N,N-Dimethylformamid.

Im Falle der Solubilisation der N-(4-Aminobenzoyl)-L-glutaminsäure wurde unter Verwendung von 300 mg N-(4-Aminobenzoyl)-L-glutaminsäure in gleicher Weise verfahren. 2 ml einer Verdünnung des Filtrats (5:1000) werden in einem 25-ml-Kolben diazotiert, gekuppelt[15] und die Extinktion nach 10 min bei 550 nm bestimmt.

2. Charge-Transfer-Bande. Die Charge-Transfer-Banden von Folsäure bzw. Tetrahydrofolsäure mit Chloranil wurden in der Weise erhalten, dass gleiche Teile von Lösungen der Folsäure bzw. Tetrahydrofolsäure ($1 \times 10^{-2} M$) und Chloranil ($1 \times 10^{-1} M$) in N,N-Dimethylformamid 30 bis 60 min nach der Mischung gemessen wurden. Als Referenz diente eine gleichkonzentrierte Chloranil-Lösung.

Summary. With the addition of N-phthaloyl-DL-glutamic acid, a metabolite of thalidomide, the solubility of folic acid rises owing to greater complexity. These results could explain the teratogenic effect of thalidomide.

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Chronic Ingestion of Rubidium without Toxicity: Implications for Human Therapy

Rubidium[1] has been found to alter the EEG and behavior of monkeys[2]. On the basis of these and other observations, as well as analogous effects with lithium, it has been suggested that rubidium might have therapeutic application in the affective disorders of humans. Since such an application might require long-term ingestion of rubidium, it was imperative to determine the effect on animals of chronic rubidium ingestion. There are several reports[3–9] indicating that repeated administration of rubidium to animals frequently ends in convulsions and death. The observations reported in this paper demonstrate the conditions under which rats can be maintained for at least 3 generations while chronically ingesting rubidium via the drinking water without deleterious effects on health.

Materials and methods. Sprague-Dawley rats, fed a normal Teklad chow, were given as their only source of drinking water a solution containing 10 mmoles each of RbCl and KCl per l of distilled water (group Rb + K). A control group was similarly supplied with a drinking water containing 20 mmoles per l of KCl (group K + K). Both groups were started on this water immediately after weaning. The drinking water was kept constant in composition for 3 generations, except as noted below.

Some animals from each group were retained for breeding. The resulting progeny were bred and became in turn the source of a 3rd generation. All of the following data refer to the 3rd generation. Some of the 3rd generation were supplied with normal drinking water after weaning; the remainder were continued on the (Rb + K) or (K + K) water. Some of the (Rb + K) animals were killed and several of their organs assayed for Rb by atomic absorption spectrophotometry[10] and for Na and K by flame photometry. A colony of rats raised under normal breeding conditions in the same facilities, approximately during the same time span, provided comparable breeding data.

Results. There were no statistically significant differences among the three groups, either with respect to weight of the litters at weaning, deaths prior to weaning, or the percentage of deaths that occurred within the year following weaning (Table I). When the (Rb + K) group was divided into 2 subgroups, 1 ($N = 20$) maintained on the normal water after weaning, and another ($N = 72$) maintained on the (Rb + K) water, it was found that the subgroups did not differ in mortality rates during the year following weaning.

Of the 5 organs examined for monovalent cation content (Table II) the liver showed the greatest, and the brain showed the least capacity to accumulate Rb. The ratio, Rb:K, averaged 0.32 for liver, 0.16 for kidney, 0.10 for lung, 0.08 for heart and 0.03 for brain.

Members of the (Rb + K) group were more excitable than members of the (K + K) group. Three of the 15 females of the former, and none of the 5 of the latter group, attacked their young. In 2 instances this took the form of cannibalization of apparently healthy young at 2 and 16 days post-partum; in the 3rd instance the female bit off $^1/_2$ to $^2/_3$ of the tails of each of her young, and repeated this attack sometime later with a second litter. The ease with which a startle response could be evoked was strikingly different for the 2 groups. In contrast to the (K + K) group a small puff of air directed at most sleeping members of the (Rb + K) group would cause them to jump violently, often hitting the top of the cage.

Table I. Comparison of breeding data for experimental, control and normal groups

Parameter	Group		
	(Rb+K)	(K+K)	Normal
Mean number per litter	8.6 (S.D. = 2.25)	10.5 (S.D. = 2.43)	10.5 (S.D. = 2.04)
Weight at weaning (g)			
Males	67	64	72
Females	53	55	62
Ratio male:female	1.1	1.3[a]	0.9
Deaths prior to weaning (%)	6.9	0[a]	7.6
Deaths within 1 year after weaning (%)	15.2	14.3	(not available)

[a] Data from 4 litters.

Table II. Range and average concentrations of sodium, potassium and rubidium in some rat organs

Organ	Range			Average		
	Na	K	Rb	Na	K	Rb
Liver	62–74	129–185	22–64	68	146	46
Kidney	149–303	144–197	22–36	191	178	28
Lung	163–340	165–321	21–34	281	269	28
Heart	212–284	285–362	19–30	245	336	26
Brain	378–506	519–753	16–24	431	653	20
Plasma[a]	141–144	4.0–4.6	0.59–0.64	143	4.3	0.62

All amounts expressed in terms of μmoles of cation per g dry weight of tissue except where indicated. [a] Amounts expressed in terms of mmoles of cation per l of plasma.

Discussion. Studies on the effects of Rb on a number of animal species led to the suggestion that its toxic action was dependent upon neural mechanisms[11]. Chronic ingestion of Rb by rats has been found to produce irritability and death 'in violent tetanic spasm'[3], audiogenic seizures[4], a tendency to convulse after handling[5], and neuromuscular irritability[6]. The incoordination and irritability observed in chicks following Rb feeding led the authors to suspect an interference with the role of K in the nervous system[9].

The marked contrast between the data reported in this paper and the prevalent experiences of others strongly suggests that there is a fairly sharp cut-off point in the parameters associated with the safety or toxicity of chronic ingestion of Rb salts. The percent of Rb in the diet, and the dietary molar ratio of Rb to K, are two parameters that are either explicitly stated, or can be estimated from other data in various publications.

Since each rat of our (Rb + K) group was fed 14 g of the Teklad chow containing 0.89% K (dry weight) and consumed an average of 35 ml of the drinking water, the K ingestion was 3.55 mmoles and the Rb ingestion was 0.35 mmoles per day. Thus the Rb content of the diet was 0.21% and the Rb:K molar ingestion ratio was 0.1.

A survey of the literature (Table III) indicates that while the relation of dose level to toxicity is erratic, there is a reasonable correlation of Rb:K molar ingestion ratio with toxicity. At moderate levels of dietary Rb[12],

[1] It should be understood that whenever rubidium, potassium or sodium is mentioned, the referenced substance was present as a salt, such as rubidium chloride.

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[12] Levels below the LD_{50}, which is given as 1.2 g/kg body weight.

Table III. Rubidium toxicity as a function of potassium ingestion

Authors	Rb in the diet (%)	Molar ingestion ratio (Rb:K)	Observations
Glendening[5] (1956)	0.01	0.017	survived for 300 days
Meltzer and Lieberman (this journal)	0.2	0.1	3 generations of healthy animals
Glendening[5] (1956)	0.1	0.16	survived; no healthy progeny
Heppel and Schmidt[14] (1938)	0.28	0.25	survived for 86 days
Follis[4] (1942)	0.5	0.3	death after 14 days
Glendening[5] (1956)	0.2	0.33	death after 80 days
Johnson et al.[8] (1968)	1.8	0.93	weight loss in 42 days, then death
Glasser and Ellis[7] (1961)	0.4[a]	1.0	failure to gain weight (20 days)
Mitchell et al.[3] (1921)	0.65	1.0	death in 10 to 17 days

[a] Estimated from concentration in drinking water, assuming that 30 to 40 ml per day were ingested.

toxicity appears when the ingestion ratio is greater than 0.1. This hypothesis was tested directly as follows. A group of 12 male Sprague-Dawley rats housed 2 to a cage was fed a K-deficient diet (Nutritional Biochemicals) and given drinking water which contained 10 mmoles/l each of KCl and RbCl. Since each rat consumed an average of 14 g of food daily and 30 ml of water, the K intake was equivalent to at least 0.085% of the diet. After 25 days 5 rats were dead. Weight gain was minimal with all rats. Ten of the 12 rats had 1 or more audiogenic seizures (induced by allowing compressed air to escape through a valve for 30 sec). At that time the surviving rats were returned to a normal diet (containing 0.89% K dry weight). Weight gain was apparent on the 7th day at which time susceptibility to audiogenic seizures disappeared. On the 15th day they were returned to the K-deficient diet. Within 2 weeks all were again susceptible to audiogenic seizures and an additional 3 rats had died. This experiment was repeated on a group of 32 rats (male and female), with the exception that they were not subjected to the compressed air stimulus. At the end of the 4th week 19 of the rats were dead. Thus it was not the Rb level per se, but its ratio to K intake, that determined toxicity.

Since the dietary requirement for K can be met in part by substituting Rb[4,7,13], the appearance of toxicity when Rb in the diet is equivalent to more than 10% of the K indicates that accumulation of Rb by tissues and organs has proceeded to a level at which another effect of Rb assumes quantitative importance. Below that level Rb either serves entirely as a substitute for K or its actions are compatible with normal cell function; above that level either its own physiological actions perturb cell function (in the same sense that an excess of K would be toxic), or new wholly pathological actions become apparent.

It is clear from the data that continued ingestion of Rb is compatible with survival and health. Although Rb was not demonstrably toxic, it was not without effect on the central nervous system. There was a general increase in excitability, as suggested by maternal attacks on the young and the ease with which the animals were startled.

Finally when the animals were made toxic by restricting the K intake to levels equimolar with Rb intake, it was observed that the resultant failure to gain weight and susceptibility to audiogenic seizures could be reversed by increasing the K intake without diminishing the Rb intake. Thus in rats Rb toxicity appears to be reversible[15].

Résumé. En vue de l'emploi anticipé du RbCl en médecine, la présente étude établit les conditions d'administration chronique et sans toxicité chez le rat. L'administration chronique étendue à 3 générations de rats Sprague-Dawley ne révèle aucun effet sur la fertilité, la gestation, le développement ou la longévité.

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Influence of Dihydroergotamine on the Lipolytic System of Isolated Dog Fat Cells

It has been shown by several workers that dihydroergotamine (DHE) antagonizes catecholamine-induced lipolysis and, at higher concentration also ACTH-induced lipolysis[1-4] in isolated rat fat cells. FAIN[2], HOTTA et al.[5] and our experiments showed that DHE was moderately lipolytic by itself in vitro. We found a maximal lipolytic activity with about $10^{-5}M$ DHE (unpublished results) in rat fat cells suspensions.

In vivo, we found that DHE does not stimulate the lipolysis in fasted rats. It was also shown[6] that DHE completely blocks epinephrine-induced increase of plasma free fatty acids.

In the fasted dog, SCRIABINE et al.[7] showed that DHE in doses of 0,125 and 0,5 mg/kg i.v. increases plasma concentration of FFA. These findings were confirmed by SIREK et al.[8] and in our laboratory. In their work, SCRIABINE et al.[7] concluded that DHE may stimulate adrenergic receptors of the adipose tissue, or activate lipolytic mechanisms beyond adrenergic receptors.

By in vitro experiments, we attempted to explain the mode of action of DHE on the isolated dog fat cells.

Methods. Preparation of isolated fat cells. Fasted mongrel dogs (5–12 kg) both sexes, anaesthetized with sodium pentobarbital (30 mg/kg) were used as donors of subcutaneous adipose tissue. The fat cells were isolated by the method of RODBELL[9], modified so that the incubation medium used for the digestion of the tissues did not contain glucose. After isolation, the fat cells were suspended in a Krebs-Ringer phosphate (KRP) solution, pH 7.4 with 4% albumin. One-ml portions of the suspension were given into 25-ml plastic vials containing 1 ml of KRP with the drugs to be tested. After a 2 h incubation at 37 °C with air, the incubation medium was analyzed for glycerol content using the method of LAURELL and TIBBLING[10]. The results are expressed as μmoles of glycerol per mmole of triglycerides in the cell suspension.

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