up to 10 days at 4-6°C with recovery of viable cells after trypsinization decreasing sharply after 24-48 hours of cold storage.

Discussion. Since cells held up to 6 weeks at 4-6°C resume growth when returned to the 34-35°C incubator, it is assumed that refrigerated monkey kidney tissue cultures do not "age" significantly during the storage period. Cultures held 3-4 weeks at incubator temperature become very granular whereas cultures stored at 4-6°C for the same interval and then returned to the incubator, have the appearance of young cells and do not show the granularity of age until an additional 3-4 weeks at incubator temperature have elapsed. It is not imperative that the nutrient media be replaced with fresh media before the cultures are refrigerated although it appears that cultures recover more rapidly if the medium is changed before the cells are stored.

The advantages of storing cells are apparent. Cultures not immediately needed can be stored under refrigeration for use at a later date. Similarly, in situations where the supply of monkey kidneys and the need of cultures do not coincide, one can prepare cultures when the tissue is available and hold them in the cold for later use. A bank of tissue cultures can be maintained for immediate service.

Perhaps one of the greatest advantages of using stored cells is that while the majority of a cell lot is refrigerated, an aliquot can be incubated and studied for the presence of simian agents, foamy agents, and non-specific degeneration before the cell lot is used for virus studies, vaccine production, or the safety testing of poliomyelitis vaccine.

Summary. Monkey kidney tissue cultures survive refrigeration at 4-6°C for periods up to 6 weeks. Cultures so treated can be returned to an incubator and maintained for 3-4 weeks at 34-35°C. Refrigerated cultures appear to retain full sensitivity to polioviruses.

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A Confirmatory Test for Mephenesin-Like Action of a Compound on Mice. (23738)

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The central paralyzing action of mephenesin, producing relaxation of the skeletal muscles in the limbs of an animal, is a unique property for agents of this pharmacologic type(1). This property, which causes loss in the ability of a mouse to right itself when placed on its back, has been employed for screening mephenesin-like compounds(2). Mephenesin also causes loss of pinna reflex and corneal reflex in mice; and by observing which reflex is lost first one may distinguish mephenesin from barbiturates, choral hydrate, and other hypnotics. Under the influence of mephenesin, the pinna reflex disappears before the corneal, whereas with other drugs two reflexes disappear in a reverse order(3). The confirmatory test reported here for the mephenesin-like action of a compound is based on the observation that a minimal paralyzing dose of mephenesin, causing the loss of righting reflex in mice, is ineffective in suppressing the Straub-Hermann mouse tail reaction due to morphine.

Methods. Male albino mice weighing 18-22 g were used. The test compound in solution or suspended in gum acacia was given to 10 animals at each dose level. Subsequently, depressive signs and symptoms, such as quietness, ataxia, loss of grasping reflex, and loss of righting reflex, were noted. Some time

TABLE I. The Influence of Certain CNS Depressants on Straub-Hermann Mouse Tail Reaction to Morphine (20 mg/kg intramusc.).

Compound	Dosage: mg/kg intraper.	Dosage: Interval before morphine	Symptoms*	Tail reaction: Mean ± S.E.	Diff.	"T"	"P"
Control				1.53 ± .10			
Mephenesin	200	0	L.R.R.	1.47 ± .17	.06	.3	.1
	250	0	"	1.07 ± .10	.46	2.9	.01
	300	0	"	.07†			
	350	0	"	.00			
Zoxazolamine	100	15 min.	"	1.33 ± .12	.20	1.2	.1
	250	15	"	.00			
Phenobarbital	50	30	Quiet	1.18 ± .15	.35	1.9	.05
	100	30	L.G.R.	.23 ± .09	1.30	8.6	<.01
	150	30	L.R.R.	.00			
Barbital	100	30	Quiet	1.03 ± .14	.50	3.3	.01
	150	30	L.G.R.	.44 ± .12	1.09	6.4	<.01
	200	30	L.R.R.	.00			
Chloralhydrate	100	15	Quiet	1.06 ± .06	.47	3.8	.01
	150	15	"	.82 ± .09	.71	5.0	<.01
	250	15	L.R.R.	.21 ± .07	1.32	10.3	"
	300	15	"	.00			
Meprobamate	150	15	Quiet	.91 ± .22	.62	2.8	.01
	200	15	L.R.R.	.00			
Dilantin	50	3 hr	Quiet	.97 ± .14	.56	3.2	"
	100	3	L.G.R.	1.05 ± .12	.48	2.8	"
	200	3	L.R.R.	.55 ± .04	.98	8.4	<.01

* L.G.R. = Loss of grasping reflex. L.R.R. = Loss of righting reflex.
† 4/10 mice showed a slight reaction toward end of one hr.

later, depending upon the duration of effect of a test compound determined previously, a challenging dose of morphine sulfate (20 mg/kg) was then injected intramuscularly. Fifteen minutes after morphine administration, the mouse tail reaction as well as the depressive symptoms were observed and recorded at 5-minute intervals for a period of 50 minutes. The numerical ratings for tail erection are those adopted by Juul(4) as follows: 1 = 45°, 2 = 90°, 3 = 180° from the table plane; 1.5 and 2.5 = intermediate reactions between 45°-90° and 90°-180°, respectively. The mean and standard errors of tail response for 10 mice are computed from the averages of 10 readings for each animal(5).

Results. As shown by the data in Table I, loss of righting reflex in mice resulted following intraperitoneal injection of 200 mg/kg of mephenesin or 100 mg/kg of zoxazolamine. At these levels, however, they did not significantly affect the mouse tail reaction to morphine. Only at higher paralytic doses was the tail response to morphine suppressed by these drugs. In contrast the morphine-induced tail reaction in mice was markedly impaired under the influence of phenobarbital, barbital, chloral hydrate, and meprobamate at sedative dose-levels and completely prevented by them at minimal paralytic dosages. A suppressive effect of Dilantin on the tail reaction was obtained also at non-paralytic doses, although the inhibition of the tail reaction was not complete with this drug even at a minimal lethal dose of 200 mg/kg. Therefore, it is possible, by the mouse tail reaction to morphine and depressive symptoms at appropriate dosages, to distinguish clearly a depressant property of mephenesin from that of Dilantin or a barbiturate-like compound.

The difference in their depressant effect at threshold and at higher paralyzing doses upon the morphine-induced tail reaction in mice indicates evidently the 2 sites or mode of actions of mephenesin or zoxazolamine on the central nervous system. It is commonly held that these drugs apparently affect the CNS structures in an ascending order. The spinal cord may be the only site to be affected by the mephenesin type of drug at low dosages.

This test, therefore, may be utilized also to determine the dosages of these compounds for their depressant effect on different parts of the central nervous system. Since high paralyzing doses of mephenesin or zoxazolamine are required to antagonize the stimulating action of morphine, it appears to support the opinion of some that higher centers above the spinal cord are involved in the mouse tail reaction to morphine(6,7).

Summary. The mouse tail reaction to morphine is not suppressed by mephenesin or zoxazolamine at minimal paralyzing dose levels. On the other hand, it is markedly suppressed by phenobarbital, chloral hydrate, meprobamate, and Dilantin at sedative dosages. The lack of a suppressive influence of an agent on the morphine-induced tail response in mice at paralyzing doses is suggested as a confirmatory test for mephenesin-like property.

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Metabolic Fate of S^{35} Administered to Rabbits as Sulfate.* (23739)

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In recent years studies making use of radiosulfur (S^{35}) have demonstrated that inorganic sulfur can be used by ruminants for the synthesis of cystine and methionine(1,2,3). These studies suggest that the extensive microbial synthesis occurring in the rumen may be responsible for the appreciable extent to which inorganic sulfur is utilized in the formation of organic sulfur compounds. Several recent studies have reported the pattern of tissue uptake of S^{35} observed in rabbits following administraiton of S^{35}-labeled sulfate (4,5,6). In the case of the last of the above cited studies, it was observed that the collaring of rabbits in such a manner as to prevent coprophagy, which apparently is habitually practiced by rabbits in the absence of a restraining device, results in an appreciable lowering of the uptake of S^{35} in the blood, liver, spleen, muscle and intestinal tissues. The present study was carried out to determine if any conversion to organic form could be detected after S^{35}-labeled sulfate was administered to rabbits.

Procedure. A dose of approximately 0.9 millicurie of S^{35} in the form of sodium sulfate plus 0.22 mg of carrier sodium sulfate was given by stomach tube to each of 4 littermate, female New Zealand white rabbits. One pair of rabbits was dosed at 10 weeks of age, and the others at 12 and 14 weeks of age. The animals were fed a commercial pelleted ration containing 0.23% total sulfur and 16.9% crude protein. The methionine content was 0.22% and there was 0.18% cystine present. During the collection period 2 of the rabbits wore plywood collars to prevent coprophagy. The collars were made of ¼-inch thick plywood. They were fitted closely about the neck by means of a rubber cushion which lined the neck opening, and had outside diameters of about 10 inches. The ex-

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