TABLE VI.
Efficacy of Streptomycin in Mice Infected with *D. pneumoniæ*. (Subcutaneous Therapy.)

Organism: *Diplococcus pneumoniæ* Type I, No. 37.
Age of Culture: 6 hours.
Infection: 0.5 cc of a 10^{-6} culture dilution in broth.
Therapy: Streptomycin or streptothricin given subcutaneously immediately after bacterial inoculation.

No. of mice	Drug	Units per dose	No. doses per day	Culture dilution	No. surviving in days 1	2	3	4	5	6	7	8	Survival %
				Therapy: A single dose.									
10	Streptomycin	50	1	10^{-6}	1	0	0	0	0	0	0	0	0
10		100	1	"	1	0	0	0	0	0	0	0	0
20		200	1	"	1	0	0	0	0	0	0	0	0
20		400	1	"	9	2	2	1	1	1	1	1	5
20		800	1	"	19	6	6	5	5	5	5	5	25
20		1600	1	"	20	20	20	20	20	20	20	20	100
10		3200	1	"	10	10	10	10	10	10	10	10	100
10		6400	1	"	10	10	10	10	10	10	10	10	100
				Therapy: A single dose.									
20	Streptothricin	200	1	10^{-6}	0	0	0	0	0	0	0	0	0
20		400	1	"	3	0	0	0	0	0	0	0	0
20		800	1	"	14	2	2	0	0	0	0	0	0
20		1600	1	"	19	11	8	5	3	3	2	2	10
				Therapy: None.									
30	Controls	—	—	10^{-6}	0	0	0	0	0	0	0	0	0
20		—	—	10^{-7}	2	0	0	0	0	0	0	0	0
20		—	—	10^{-8}	4	0	0	0	0	0	0	0	0
20		—	—	10^{-9}	6	0	0	0	0	0	0	0	0

seems to be due to a histamine-like substance present in the toxic preparations. To date, sufficient quantities of streptomycin have not been available for extensive toxicological and pharmacological studies. Until such studies are completed, the value of this substance for the treatment of bacterial disease in animals and man cannot be fully evaluated. Although streptomycin appears to have a number of advantages over streptothricin, the latter is much more effective against fungi.

Summary. Streptomycin seems to possess some advantages over streptothricin particularly from the point of view of toxicity and its greater action against certain gram-negative and gram-positive bacteria *in vivo*. Although certain batches of streptomycin appear to have the same order of toxicity as streptothricin, the findings suggest that under proper conditions a relatively non-toxic preparation can be obtained. Streptothricin seems to be much more effective *in vitro* against pathogenic fungi than streptomycin.

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Effect of Feeding Dried Milk on Production of Liver Cancer by *p*-Dimethylaminoazobenzene.

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Dietary factors greatly influence the formation of liver cancer by *o*-aminoazobenzene or *p*-dimethylaminoazobenzene (butter yellow). Experiments have shown that the incidence of liver cancer in rats caused by these chemicals is definitely reduced when the basal diet consists of wheat,[1] rye,[2] or millet seed[3] instead of rice.

[1] Ando, T., *Gann*, 1938, **32**, 252.

[2] Maisin, J., *Bull. Assoc. franc. p. l'étude du cancer*, 1940, **29**, 6.

TABLE I.
Incidence of Hepatic Tumors in Rats Fed Different Amounts of Dried Whole Milk.

Diet	No. of animals used	No. of days fed	Liver findings*					Incidence of liver cancer, %
			—	±	+	++	+++	
Butter yellow and rice	39	100-200	0	0	5	23	11	100
Butter yellow and rice and 6% Klim	20	150-163	2	0	0	14	4	90
Butter yellow and rice and 15% Klim	20	154-198	5	3	4	4	4	60
Butter yellow and rice and 20% Klim	20	132-227	10	0	2	4	4	50

* — indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma, or both; ++ indicate extensive liver cancer without metastases; +++ indicate extensive liver cancer with metastases.

It is also known that the addition of yeast,[4,5,6] or liver[7] to a rice diet increases resistance to this type of tumor formation.

It has also been reported that *p*-dimethylaminoazobenzene will fail to produce liver cancer in rats maintained on a rice diet supplemented with either casein and riboflavin,[8] casein and B vitamins,[9] or cystine and choline.[10]

The experiments described in this report were undertaken to determine the effect of dried milk feeding on the incidence of hepatic tumors in rats after feeding *p*-dimethylaminoazobenzene.

Experimental. In the present study, dried whole milk* was chosen because of its high casein and riboflavin content (28.0% fat, 26.7% milk protein and 1.6 mg riboflavin per 100 g).†

Three groups of 20 rats each (125 to 150 g body weight) were maintained on a basal diet of unpolished rice mixed with 6, 15, and 20% of dried whole milk, respectively. A control group of 50 rats was kept on unpolished rice alone. All animals received 3% *p*-dimethylaminoazobenzene in cottonseed oil in the proportion of 20 cc of the solution per kg of ration noted above. All of the 4 groups of rats received a small amount of fresh carrots daily. Unlimited water was allowed. Feeding on the various diets was continued for about 200 days; all rats then living were sacrificed and examined.

The results obtained from this study are summarized in Table I. Rats that died before the period of 100 days are not included in the table. In all cases the gross diagnosis was confirmed by microscopic examination. Rats used in this study were of the Sherman stock.

As may be seen in Table I, the addition of 15 and 20% of dried whole milk to unpolished rice had a distinct protective effect, though not absolute, against the pathological changes leading to the development of liver cancer. Among 20 rats fed 20% dried whole milk, 10, or 50%, had grossly and histologically normal livers; the remaining 10 rats showed numerous large and small tumor nodules with or without metastases in the mesentery and omentum. On the other hand, the animals fed the azo dye-rice diet without Klim developed typical liver cancer in all cases in comparatively shorter periods of time. The addition of 6% Klim to the butter yellow-rice diet, however, had practically no inhibitory effect upon the production of liver cancer.

Animals on the butter yellow-rice diet containing 20% dried whole milk consumed from 7 to 9 g of the food daily. Therefore, a rat

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9 Miller, J. A., Miner, D. L., Rusch, H. P., and Baumann, C. A., *Cancer Research*, 1941, **1**, 699.

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* Klim, manufactured by The Borden Co., New York.

† The author is indebted to Dr. Raymond Hertwig of The Borden Co. for information regarding the composition of the powdered whole milk used.

consumed approximately 0.4 g of casein and 26 μg of riboflavin daily.

The inhibitory effect of dried whole milk feeding upon the production of liver cancers is distinctly less than that of the combination of purified casein and crystalline synthetic riboflavin, or the feeding of a diet of unpolished rice containing 15% of brewers' yeast (50% against 93 and 100% protection, respectively, at 200 days). Part of the difference in the protective effect in these cases may be due to variations in the vitamin levels (daily ingestion of 26 γ against 200 and 84 γ riboflavin, respectively).

In order to eliminate the possible tumor-promoting activity of the high-fat content of Klim the following experiment was undertaken. Forty young adult rats were maintained on the butter yellow diet of 85% rice and 15% of dried skimmed milk (Powdered Skimmed Milk, The Borden Co., New York.) It contained 1.0% fat, 36.5% protein, and 1.9 mg riboflavin per 100 g.

The results showed that the reduction in the amount of fat content in the diet did not increase the protective action of dried milk against liver cancer formation. Of the 34 rats which died or were sacrificed between 150 and 250 days after the beginning of the experiment, 14, or 41%, had grossly and microscopically normal livers; and 20, or 59%, had liver cancer, both cholangioma and hepatoma.

The majority of the animals fed the butter yellow-rice diet with dried whole milk gained weight and presented a healthy appearance throughout the experimental period.

Effect of Adding Dried Whole Milk to the Basal Rice Diet Following Periods of Feeding a Butter Yellow-Rice Diet. A recent study[11] showed that a majority of rats fed a butter yellow-rice diet for 60 days developed cirrhosis of the liver. At this time, 20% of the livers also showed adenomatous hyperplasia of the bile ducts with beginning malignant transformation into cholangiomas, or early hepatoma formation. The withdrawal of butter yellow from the diet at this stage did not prevent the subsequent development of liver cancers. However, this precancerous condition (cirrhosis of the liver) was abolished to a large extent by a rice diet containing 15% yeast.[11]

The succeeding paragraphs deal with attempts to ascertain whether a destructive action against experimental liver tumors can be accomplished by the addition of dried whole milk to the basal diet after the formation of hepatic cancers, or precancerous states, resulting from the previous ingestion of *p*-dimethylaminoazobenzene.

Following the procedure elsewhere described,[11] the author maintained rats on a butter yellow-rice diet for definite periods of time. Then the carcinogen was removed from the food and feeding was continued on the basal unpolished rice containing a supplement of 15% dried whole milk, until the animals either succumbed or were sacrificed.

The results are presented in Table II.

The data in Table II show clearly that dried whole milk feeding produced a striking inhibition of liver cancer development. In 20 rats which received the preliminary feeding with butter yellow for 62 days, followed by rice-dried whole milk diet without butter yellow for 113 to 168 days, 18, or 90%, had smooth, practically normal livers. In contrast, of animals fed the butter yellow-rice diet for the same period, followed by rice alone, only 12% had histologically normal livers.

If the preliminary feeding with butter yellow exceeded 88 days, liver cancer resulted in almost 100% of the cases. These tumors also showed metastases in the mesentery and omentum, even though the dietary anticarcinogen (powdered whole milk) was fed for long periods of time subsequent to butter yellow ingestion.

Aspiration biopsies were performed on the livers in 34 cases to establish the presence of liver cirrhosis and tumors. About 60% of the livers of rats maintained on butter yellow-rice diet for 60-70 days gave evidence of cirrhosis, while about 75% of the livers of rats maintained on butter yellow-rice diet for 100 days or longer gave positive evidence of tumor formation.

Summary. 1. The therapeutic action of dried whole milk upon liver cirrhosis and cancer has been investigated. The liver changes were

[11] Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1942, **2**, 453.

TABLE II.
Results of Adding Dried Whole Milk Following Periods of Butter Yellow-Rice Diet, on the Production of Liver Cancer.

No. of animals used	No. of days fed on butter yellow and rice	No. of days fed rice and Klim diet subsequent to butter yellow-rice diet	Liver changes*				
			—	±	+	++	+++
20	62	113-168	18	0	0	0	2
27	88	100-250	1	0	0	5	21
14	124	82-254	0	0	2	6	6

* See Table I for explanation of symbols.

induced in rats by feeding unpolished rice and *p*-dimethylaminoazobenzene. 2. The production of liver cancer in rats fed *p*-dimethylaminoazobenzene was partially inhibited by the daily ingestion of dried whole milk (50% protection at 200 days). 3. Liver cirrhosis produced by *p*-dimethylaminoazobenzene has been treated successfully by a rice diet containing 15% dried whole milk. (Based on previous evidence of the high incidence of liver cirrhosis in rats produced by *p*-dimethylaminoazobenzene-rice diet.[11]). 4. Once adenomatous hyperplasia of bile ducts, cholangioma, or hepatoma had been established in the livers, these benign and malignant tumors could not be destroyed by ingestion of the rice-milk diet.

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Effect of Malonate, Fumarate, Succinate, and Citrate on Synthesis of Acetylcholine *in vitro*.*

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The increased synthesis of acetylcholine *in vitro* under aerobic conditions is greater in the presence of serum and spinal fluid obtained from healthy humans than from patients with myasthenia gravis.[1,2,3] Since the synthesis of acetylcholine *in vitro* is increased in the presence of glucose and pyruvate[4] the following experiments were designed to ascertain whether or not possible intermediates of glucose and pyruvate metabolism modify the synthesis of acetylcholine.

Method. The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[4] with minor modifications.[2] Varying amounts of sodium succinate, sodium fumarate, sodium malonate, sodium citrate, and sodium oxalate were added to mixtures containing 100 mg minced fresh frog brain, 1 cc serum or spinal fluid, 3 mg physostigmine salicylate, and 2 cc Ringer's solution. The pH of the mixtures was adjusted to 7.4. Identical mixtures without the salts of these organic acids served as controls. The mixtures were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of free and total acetylcholine synthe-

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3 Torda, C., and Wolff, H. G., *Science*, 1944, **100**, 200.

4 Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Bioch. J.*, 1936, **30**, 1668.