

TABLE I

Length of time B.Y. diet (days)	Supplement		No. rats assayed	No. rats with liver cancer	Per cent. rats with liver cancer	Riboflavin liver levels micrograms/gram fresh liver (most normal tissue)
	Material	Am't/rat/day				
110-130	0	0	35	34	97	10.5
117-126	Riboflavin	5 mg	16	13	81	18.2
118-126	Nicotinic acid	3 "	15	13	87	20.0
115-145	Riboflavin	5 "	17	8	47	26.6
	Nicotinic acid	3 "				
124	Riboflavin	200 micrograms	8	6	75	21.2
	Nicotinic acid	3 mg				
120	Corn oil	400 mg	5	5	100	11.5
120	Riboflavin	200 micrograms	10	8	80	18.2
	Corn oil	400 mg				
120	Casein	2 gm	10	8	80	13.8
	Corn oil	400 mg				
120	Riboflavin	200 micrograms	10	0	0	28.4
	Casein	2 gm				
	Corn oil	400 mg				
149	Casein	2 gm	17	12	71	17.1
150	Riboflavin	200 micrograms	16	1	6	26.0
	Casein	2 gm				
160-210	Yeast (20-40)	1.5 gm	22	0	0	28.2
Rats fed normal diet, average						
Rats fed basal diet of brown rice and carrot, average						
Liver cancer which results from the feeding of butter yellow with basal diet, average						

NOTE. The use of casein in these experiments was suggested by a joint study in progress with Professors Vincent du Vigneaud and Dean Burk, of Cornell University Medical College. With them various factors that might be involved in addition to riboflavin are under investigation, particularly the sulfur-containing amino acids.

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INHIBITION OF LIPASE ACTIVITY IN RAW MILK

ONE of the most objectionable flavors which occurs occasionally in milk is the so-called rancid flavor. This flavor is due to a partial hydrolysis of milk fat by lipase. The presence of free fatty acids of lower molecular weight in milk, and especially that of butyric, is responsible for this flavor.

Ordinarily, when cows have an access to green feed the problem of lipolysis in milk is not of great concern. However, in the winter time, when cows are on dry feed, the milk industry is confronted with a serious problem of spontaneous lipolysis in milk from cows that are usually late in lactation. The milk will become rancid without any apparent activation measures, such as homogenization, usually on standing four hours or longer after milking, unless the milk is pasteurized in time to inactivate the lipase.

In the course of a study of the factors responsible for the wide variation in the concentration of naturally active lipase in milk we were impressed with the phenomenon of the absence of lipolysis while the milk

is in the udder, no matter how high the concentration of lipase in milk might be potentially. The speculation upon this feature, together with some other irregularities in the activity of lipase, led us to the discovery which, we hope, will give a sounder basis for further study of lipase activity in milk, and, in some measure, will be of immediate value to men in industry and of interest to biochemists.

We employed the measurements of surface tension of milk (as well as of pH and titratable acidity when it was feasible) to determine the rate of lipase action. Briefly, our data on milk containing a naturally active lipase, obtained from several cows at various times, show:

1. After milk is drawn from the udder the lipase is activated by cooling of milk. As long as the milk is kept at a temperature of the body or near to it the enzyme remains relatively inactive almost for the duration of the life of milk. In fact, the rate of lipase action is so negligible even at temperatures between 30°-20° C., that milk relatively high in lipase will still not become significantly rancid unless the milk is cooled during aging to a lower temperature. The critical cooling temperature appears to be between 20°-15° C. and the rate of lipase action is increased with progressive cooling to lower temperatures. The most important feature of the activation of milk lipase by cooling is that once the milk has been cooled, the activity of lipase is not materially affected whether the milk is aged in the cold or rewarmed immediately to 20°, 30°, or 37° C. after cooling and aged at those temperatures. Actually, the velocity of lipase action on milk fat is even slightly greater when cooled milk is rewarmed to 25° C. and aged at 20°-25° C., provided the milk is of low bacteria count. The rapid growth of certain bacteria and the development of acid, we know,¹ will hinder the speed of lipolysis. It is also clear that the

¹ N. P. Tarassuk and F. R. Smith, *Jour. Dairy Sci.*, 23: 1163-1170, 1940.

physical state of fat in milk will not explain the activation of lipase by cooling. Our data indicate that the most plausible hypothesis for the acceleration of the lipase activity in milk by cooling is to be found in the effect of cooling on the permeability to lipase of the adsorption "membrane" surrounding the fat globules. A mild, churning-like agitation of milk will activate the enzyme without cooling of milk. The addition of formaldehyde to milk and aging will also increase considerably the rate of lipase action without the necessity of cooling. The addition of formaldehyde to well-cooled milk has no effect on the rate of lipase action. The ineffectiveness of formaldehyde as a milk lipase inhibitor was shown by Palmer in 1922.²

2. The holding of milk at 32°-37° C. for 1 to 3 hours immediately after it leaves the udder exerts a profound retarding effect upon the activity of lipase even though the milk is cooled afterwards. Our results show that by holding for 2½ hours at 33° C. the development of perceptible rancidity was postponed for over 30 hours in milk which otherwise became strongly rancid in 12 hours. The retarding effect is inversely related to the concentration of lipase and is progressively increased up to about 3-3½ hours of holding.

It is expected that a detailed paper of this study will be published in the near future. It is becoming increasingly evident that in a study of lipase activity in milk a knowledge of the temperature history of the milk from the time it leaves the udder is essential.

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THIURAM SULFIDE FOR TURF DISEASES

APPLICATIONS of mercury fungicides are considered necessary for the maintenance of desirable bent turf in sections of the country where turf diseases are troublesome. The war has caused such a tremendous increase in the prices of mercury that the cost of these materials has become exorbitant. This fact has occa-

sioned an increase in the work on the testing of materials for their fungicidal properties. More than 100 chemicals were tested during the past year for the control of turf diseases. Some of the thiuram sulfide compounds have shown considerable promise. Of these, tetramethyl thiuramdisulfide (known commercially as TUADS, Thiurad, and DuBay 1205-U) has been the most effective to date. Previous investigators have found it to be valuable as an insecticide¹ and also as a fungicide in the control of *Venturia inaequalis* (Cke.) Wint. on apple.²

Tetramethyl thiuramdisulfide was tested on the turf garden at the Arlington Experiment Farm, Arlington, Virginia, and on the two nearby golf courses for the control of both brownpatch (causal organism, *Rhizoctonia solani* Kühn) and dollarspot (causal organism, *Sclerotinia homoeocarpa* Bennett) on bent turf.

The experiments were conducted on three different strains of creeping bent, and no injury to the turf was observed at the rates used. The material was mixed with sufficient dry sand to serve as a carrier, broadcast evenly over the area and watered in lightly. It was applied at weekly intervals, during the summer months for the control of brownpatch, and during the spring and fall months for the control of dollarspot.

This season's applications of the chemical at the rate of 4 ounces to 1,000 square feet effected complete control of both diseases, whereas the untreated plots were 70 per cent. infected. In these series the turf was superior to that on the plots which had received treatments with mercury fungicides. Lighter applications were tried for the control of dollarspot. A 2-ounce rate gave just as effective control as a 4-ounce rate when repeated treatments were employed. Where the rate of application was reduced to 1 ounce to 1,000 square feet from 7 to 15 per cent. of the area became infected.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

NEW PARAFFIN-RESIN INFILTRATING AND IMBEDDING MEDIA FOR MICRO-TECHNIQUE

HERETOFORE the writer has used a paraffin, bayberry wax, rubber mixture for both infiltration and imbedding of tissues. The media herein described are prepared from paraffin of melting range 56°-58° C. and from a water-white hydrocarbon resin, LX-291, produced by the Neville Company, Pittsburgh, Pa. Preliminary advantages are that the ingredients are easier to obtain, the media are simpler to make, are clear and

white and can be more easily filtered. The chief technical advantage is that the solidified media suggested here differ from each other appreciably in hardness at a given temperature, but not essentially in melting range. Consequently, excellent sections, which ribbon well, of any desired thickness below about 20 µ, can be cut at room temperature.

The compositions of the paraffin-resin media are indicated here by the percentage by weight of resin in the mixtures. Hardness of the media increases as

² L. S. Palmer, *Jour. Am. Chem. Soc.*, 44: 1527-1538, 1922.

¹ H. G. Guy, Univ. of Del. Exp. Sta. Bull. No. 206, 1937.
² H. B. S. Montgomery and M. H. Moore, *Jour. Pomol.*, 15, 253-266, 1938.