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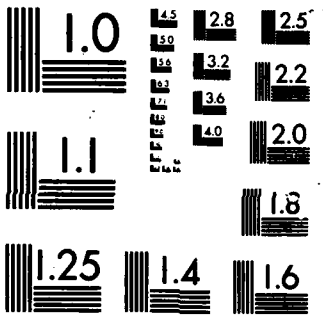

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AUTHOR (S)

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The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

ambient temperature, hypothermia, myristoyl glycerol, palmitoyl glycerol, mouse plasma lipids, mouse lymph, saturated fat, telemetering devices, toxicity, unsaturated fat

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Palmitoyl glycerol and myristoyl glycerol are toxic when fed to mice. The symptoms are hypothermia, death, and poor growth by the survivors. The toxicity is greatly exacerbated at low ambient temperatures, and is prevented if the diet is supplemented with small amounts of unsaturated fatty acids. Lymph and plasma lipids from mice fed palmitoyl glycerol contain more than 75% saturated fatty acids. The mechanism of the toxicity is not understood, but may involve the alteration of some membrane lipids so that a vital function is impaired.

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U. S. Army Research Office -- Grant Number DAAG29-81-K-0044

Effect of Ambient Temperature on the Toxicity of Palmitoyl Glycerol

Final Report

Problem Studied:

At the start of the funding of the proposal, we had shown that when palmitoyl glycerol was fed to weanling mice, about 75% of the animals died within a few days and those that survived grew poorly. The only pathological lesion associated with the toxicity was a marked inflammatory reaction in about 70% of those mice that succumbed. Adding small amounts of either oleate or linoleate to the diet would protect against the toxicity. There were no abnormalities in either the amount of diet consumed or its rate of absorption. Similarly, there were no differences between those fed the palmitoyl glycerol diet with or without safflower oil (containing 78% linoleic acid) in the rate of oxidation of [^{14}C]palmitate or oxidative phosphorylation by liver or heart mitochondria. Finally, there appeared to be a pronounced age effect, with the younger animals being more susceptible.

We sought the answers to three questions:

1. Was the immediate cause of death related to the lung lesions?
2. What was the mechanism by which palmitoyl glycerol was toxic?
3. What was the mechanism by which the unsaturated fat protected against the palmitoyl glycerol toxicity?

Although we have made considerable progress, the definitive answers to the last two questions remain.

Summary of Important Findings:

1. Myristoyl glycerol is as toxic as palmitoyl glycerol and is equally protected by linoleate or oleate supplementation.

2. An additional symptom of the toxicity was recognized. This was the appearance of a pronounced hypothermia for all animals fed the palmitoyl glycerol diet.

3. Ambient temperature played a major influence on the appearance of the toxic syndrome. For example, 15 of 28 mice fed palmitoyl glycerol and housed at temperatures below 20°C died; in contrast, death occurred in only one mouse fed the same diet but housed at a temperature of 25°C or higher.

4. There were no differences in the degree of inflammatory reaction in the lungs of the mice housed at the various temperatures, showing that this lesion was not the immediate cause of death. In retrospect, we should have realized this because 30% of the mice that died showed no lung abnormalities. Because of its sudden onset and the absence of a pathological lesion, it seems likely that the immediate cause of death must involve a vital organ that would not appear abnormal on necropsy. One possibility would be cardiac arrest.

5. It is well known that the sodium-potassium ATP pump is materially inhibited at low temperatures. Thus it seemed possible that the immediate cause of death could be cardiac arrest caused by an increase in serum potassium. Preliminary measurements of serum potassium in mice fed palmitoyl glycerol seemed to show an elevated serum potassium, although the variation was high. Because the cutoff of the potassium pump would be apt to be a threshold event, it was not unexpected that we might encounter a high variability. Clearly, what was needed was a method to follow the heart rates and body temperatures of the mice on a continual basis.

6. With the help of Dr. Tom Wollcott of the Department of Marine, Earth and Atmospheric Sciences, a telemetering system was designed that would enable the continual recording of body temperature and heart rate of each of 30 mice simultaneously. A prototype was developed and tested and we are currently testing the final stages of the complete system. The system consists of a radiotransmitter that is implanted in each mouse. The electrical signal to the heart is detected and every eighth pulse is transmitted as a square wave, thereby giving the heart rate. The width of the square wave is proportional to body temperature. The radio signals are received by a receiver mounted in each cage with its output hard wired to a computer. Since the cages are metal, the signal from each mouse is shielded from its neighbor. Two problems with the current design of the implantable device are its weight (about 2 g) and the limited life (about 2 weeks) because it is powered by a watch battery. Because of the general utility of such implantable devices for recording physiological measurements in small animals, the Department of Electrical and Computer Engineering is attempting to design a single chip, based on our unit, that will achieve the same end but be much lighter and have a longer life.

7. Considering the consistency of the observed hypothermia, the question concerning the mechanism of the toxicity may be rephrased to "By what mechanism does the palmitoyl glycerol diet produce hypothermia?" We have considered several possible explanations for this. The first of these was that a hypothermic peptide (i.e., neurotensin or bombesin) could be involved. There were several factors that led us to consider this as a possibility. First, it has been shown by Bissette et al. (Proc. N. Y. Acad. Sci. 400:268-282, 1982) that neurotensin injected intracisternally

produces a hypothermia similar to the one we observe on feeding the toxic diet. Moreover, consistent with our observations, hypothermia did not occur unless the ambient temperature was reduced. Additionally, the neurotensin hypothermia could be prevented if prostaglandin E_2 was injected intracisternally. Since linoleate, a prostaglandin precursor, was the most effective unsaturated fatty acid in preventing the palmitoyl glycerol toxicity, the notion of the involvement of a hypothermic peptide was even more attractive. Finally, it was shown by Rosell (ibid., 183 197) that the ingestion of fat caused the release of neurotensin from the gastrointestinal tract. Although peripherally injected neurotensin did not produce a hypothermia, we nevertheless examined the levels of circulating neurotensin in mice fed the palmitoyl glycerol diet with or with safflower oil by radioimmunoassay. We found no difference between the two diets, indicating that plasma neurotensin levels were not apt to be raised by the diet. We further confirmed this by showing that the administration of fairly large amounts of antibodies against this hormone had no effect on the toxicity of palmitoyl glycerol. We have thus concluded, perhaps expectedly, that the toxicity of palmitoyl glycerol bears no relation to the plasma levels of neurotensin.

8. To further test the notion of neurotensin involvement, we injected prostaglandin E_2 intracisternally into mice fed palmitoyl glycerol with or without safflower oil. This hormone had no effect on the toxicity of palmitoyl glycerol. Additionally, neurotensin levels of the hypothalami of mice fed the toxic diet were no different from those fed the palmitoyl glycerol diet protected with safflower oil.

9. Bissette et al. (loc. cit.) had shown that giving thyroid-releasing hormone (TRH) reversed the hypothermic effects of neurotensin. We therefore examined the effects of TRH in reversing the hypothermic effects of palmitoyl glycerol. Intraperitoneal injection of TRH did reverse the hypothermia of palmitoyl glycerol, but only for a short time. However, TRH has such broad physiological effects that we do not consider this as strong evidence for hypothermic peptide involvement. Thus, in balance we have rejected, for the moment at least, the idea that increased release and/or synthesis of neurotensin is associated with the toxicity of palmitoyl glycerol.

10. Since feeding palmitoyl glycerol produces the toxic effect, it became of importance to ascertain the lipid composition of the intestinal lymph. Clearly, cannulation of a lymph vessel as normally carried out with many large animals was not feasible with a mouse weighing 10-15 g. By sampling the lymph at the mesenteric lymph node, we were able to carry out the analyses on pooled samples from about 3 animals. The results of this study showed that the palmitoyl glycerol was metabolized, in that the monoglyceride fraction was only slightly elevated compared to animals receiving a chow diet. Lymph from weanling mice contained a lower proportion of triglycerides and a higher proportion of free fatty acids

and cholesterol esters than that from adult mice or other species. The lymph from mice fed palmitoyl glycerol without or with safflower oil showed even higher proportions of these lipids than the chow-fed controls. The cholesterol ester fraction was greater and the triglycerides lower in lymph derived from animals fed palmitoyl glycerol alone versus those given the safflower oil supplement. This constitutes the first significant difference in lipid composition that can be correlated with the toxic effects.

11. The fatty acid composition of the lymph from mice fed the two experimental diets was not materially different for any of the lipid fractions except for the triglycerides. When safflower oil was fed, the linoleate level in this fraction was higher as might be expected. What was striking was the degree of saturation of all of the lipids. Except for the phospholipids, that contained about 72% saturated fatty acids, the level of total saturated fatty acids exceeded 80%. At the body temperature of a normal mouse, lipids this saturated would be expected to be solid.

12. In the blood plasma, the triglycerides were higher and the cholesterol esters lower when the animals received either of the two palmitoyl glycerol diets as compared to weanling mice fed chow. Although the monoglyceride fraction in the plasma from mice fed palmitoyl glycerol, with or without safflower oil, was higher than when chow was fed, this lipid fraction comprised only 9% of the total, thus confirming what seemed apparent from the lymph studies--that palmitoyl glycerol, per se, was not the toxic compound at the cellular level.

13. In contrasting the fatty acid composition of the plasma lipids from the two palmitoyl glycerol diets, once again major differences were found in the cholesterol esters. When palmitoyl glycerol was given without safflower oil, the level of palmitate was significantly elevated over that observed when the diet was supplemented with safflower oil or when chow was fed. This resulted in a level of total saturated fatty acids in the cholesterol ester fraction of 74%, in contrast to a level of 46% or less in the supplemented or chow-fed groups. This difference was compensated for by a reduction of linoleate in the palmitoyl glycerol-fed mice. This change in fatty acid composition correlates with the gross effects of the diets on mouse survival.

14. Since oleate, as well as linoleate, is a protective fatty acid, we carried out similar studies with the palmitoyl glycerol diet supplemented with oleate. As with safflower oil supplementation when the oleate supplement was given, the palmitate level of the cholesterol ester fraction of the plasma was not elevated, but this time the linoleate remained low (4%) and the oleate level rose. Thus it would appear that the toxicity may be related to the level of saturated fatty acids in the cholesterol ester fraction of the plasma.

15. Small and coworkers (J. Lipid Res. 23:28, 1982) have shown that chylomicrons of rats fed tripalmitin containing 75% total saturated fatty acids are of a normal, spherical shape and are liquid at body temperature. On cooling below 23°C, they begin to crystallize and become oblong in shape. They do not return to the normal spheres until warmed to 53°C. Since the lymph chylomicrons from the mice fed palmitoyl glycerol are even more saturated and the temperature at which crystallization began to occur corresponds to the ambient temperature below which mortality ensues, it seemed possible that the chylomicrons of the plasma might be crystallizing in some vital organ, resulting in mortality. We have examined the structure of the plasma chylomicrons by electron microscopy, and even at low body temperatures they were found to be spherical. We have therefore concluded that structural alteration of chylomicrons is not related to the immediate cause of death.

16. The current hypothesis to account for the toxicity of palmitoyl glycerol is that the lipids of some plasma membrane domain, where an essential metabolite is transported in or out of the cell, has been altered such that the function is impaired. Most recent findings suggest that the thyroid could be so affected. Adding small amounts of propylthiouracil to the diet greatly exacerbates the effect of palmitoyl glycerol. Additionally, when the palmitoyl glycerol diet is supplemented with safflower oil, plasma levels of both thyroxin and triiodothyronine are about three times that seen when safflower oil is not given.

17. Finally, we wish to thank the Army Research Office for the generous support given us over the past seven years. Even though we have not sought additional support from A.R.O., we can assure you that this work will continue.

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