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Received July 22, 1957. P.S.E.B.M., 1957, v96.

Effect of Aureomycin on Hepatic Utilization of Labeled Body Fat in Rats.* (23475)

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Rats fed Aureomycin and given a tracer dose of $CH_3C^{14}OONa$ orally or parenterally 24 hours later have more C^{14} incorporated in hepatic fatty acids than control rats not treated with the antibiotic(1). In order to investigate the mechanism responsible for the increased radioactivity, we have studied the effect of administration of Aureomycin on mobilization of labeled body fat to the liver and on its subsequent accumulation in this organ. As part of this investigation we have made a 4-day balance study of orally administered C^{14}-tripalmitin in normal rats.

Materials and methods. Adult, male Sprague-Dawley rats were paired by weight and kept on Purina Laboratory Chow *ad libitum*. In order to label the body fat, a daily dose of 25 mg of carboxyl-labeled C^{14}-tripalmitin, contained in 1 ml of olive oil, was fed by stomach tube to each rat for 4 days. During this period and the subsequent day food was allowed *ad libitum*. The radioactive tripalmitin was not fed on the fifth day so that disappearance of absorbed labeled fat from the blood stream would be complete. On the sixth day 5 mg of Aureomycin (Lederle) were given by stomach tube to a member of each pair of rats. After administration of antibiotic the rats were fasted 24 hours and killed by decapitation. Control rats were treated similarly except that water was given instead of Aureomycin. Feces excreted during the first 5 days were pooled for each rat. Fecal samples of the sixth day were combined with the contents of the gastrointestinal tract of each animal. Immediately after decapitation of the animals the organs and tissues to be analyzed were placed in alcoholic KOH. After digestion and saponification of the samples chemical and radioactivity determinations of total fatty acids were made by procedures described previously(2).

Results. The livers of rats treated with Aureomycin contained fatty acids in amounts similar to those in the control group. The specific activity and total activity of the hepatic fatty acids of both groups also were similar. These data are given in Table I in the form of an average and standard deviation for each of the 2 groups. Examination of the data on the basis of each pair of rats also proved that there was no consistent significant difference between experimental and control animals.

In a similar manner, analyses of fatty acids of other tissues revealed no significant differences in amount or radioactivity between the 2 groups. Carcass total fatty acids of antibiotic-treated animals contained 32.5% (S.D. = 3.9) of the absorbed activity; those of controls contained 31.3% (S.D. = 5.8). Results of analyses of intestinal fatty acids were more variable and ranged from 0.78 to 2.04% of the absorbed activity in the experimental group and from 0.81 to 1.60% in the control group.

A 4-day balance period in all rats before administration of antibiotic resulted in an average absorption value of 88.1% (S.D. = 3.2) of the tripalmitin fed.

* This paper is based on work performed partly under contract with U. S. Atomic Energy Com.

† Work done while on a medical student research fellowship from National Fn. for Infantile Paralysis.

TABLE I. Amount and Radioactivity of Hepatic Fatty Acids of Aureomycin-Treated and Control Rats.

	Experimental		Controls	
	Avg	Stand. dev.	Avg	Stand. dev.
Wt of liver (g)	7.45	.95	7.18	1.18
Total fatty acids				
% of wet wt of liver	3.75	.96	3.68	.50
Specific activity (cpm/mg fatty acid)	26.52	5.32	23.55	4.32
Total activity (total cpm/liver)	7130	1400	6120	1300
Total activity (% of total body counts × 100)	25.3	3.9	22.8	5.1

All except one pair of animals showed a net loss in weight, ranging from 13 to 27 g, during the experimental period. This was due largely to the 24-hour fasting period immediately prior to sacrifice.

Discussion. No excessive amount of fat had accumulated in the rats given Aureomycin and killed after a 24-hour fast. This problem is of interest in view of conflicting reports on effects of antibiotics on liver lipids (3-5). Aureomycin was observed to cause reversible fatty metamorphosis in the liver of man(6) and to be antilipotropic in rats over a period of 15 days(7), but it was reported by György *et al.*(8) to exert lipotropic action in rats maintained on choline-deficient diets for relatively long periods of time. Since our animals received only one dose of Aureomycin and lived only 24 hours after administration of the drug, gross changes in liver lipids would not be expected. However, experiments reported previously(1) demonstrated increased amounts of C^{14} in liver fatty acids of rats given Aureomycin and fed a tracer dose of C^{14}-sodium acetate 24 hours later. These findings cannot be explained in terms of increased mobilization to the liver or decreased utilization by the liver since, in the present experiments, similar C^{14} activity was found in hepatic fatty acids of both experimental and control rats, the body fat of which had been labeled previously with C^{14}-tripalmitin. That excessive mobilization from carcass stores of fat had not occurred is indicated also by the observation that carcass total fatty acids of both groups had similar amounts of C^{14} activity. Because of the large amount of C^{14} in the carcass compared to liver, it is possible that this measurement lacked the sensitivity required to detect a significant difference between the 2 groups. Our results are in accord with the hypothesis that the observed immediate effect of Aureomycin is on lipogenesis.

The 4-day fat balance study showed that from 7 to 19% of the activity fed was recovered in the feces and intestinal contents, indicating satisfactory absorption in the presence of a relatively large amount (1 ml) of oil administered. Our results agree with those of Hoagland and Snider(9) who observed that about 15% of the tripalmitin fed in 5 or 10% solution in olive oil was excreted. Values of absorption ranging from 57 to 92% for labeled triolein were reported by Bergström *et al.*(10) in 24-hour experiments in rats given one dose of the radioactive oil. Bergström and co-workers(11) also reported that stearic acid transesterified with corn oil was absorbed to the extent of 95% of the amount fed.

Of the radioactivity absorbed only from 20 to 40% was found in the combined tissues analyzed. Since these included the entire body except the head, 60 to 80% of the activity absorbed had disappeared from the body fat through metabolic conversion to CO_2 and other compounds.

Summary. Administration of 5 mg Aureomycin to rats, the body fat of which had been labeled previously with C^{14}-tripalmitin did not result in accumulation of excessive C^{14} activity in hepatic fatty acids. The amount and radioactivity of total fatty acids of carcass and of intestine were likewise similar in experimental and control animals. These findings are discussed in relation to the observation that Aureomycin causes an increase in

incorporation of C^{14}-acetate into hepatic fatty acids of rats. A 4-day metabolic balance study showed that from 81 to 93% of the fed tripalmitin was absorbed.

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Received July 22, 1957. P.S.E.B.M., 1957, v96.

Studies on Variation in Virulence of Poliomyelitis Virus II. Role of Host Cells and Their Maintenance Medium. (23476)

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In developing strains of avirulent viruses which are satisfactory for immunization, it is important to find out whether there are any environmental factors which can affect the viral virulence. In a previous paper(1) a mouse avirulent variant, (now designated as LH-a), isolated from the mouse adapted Type III poliovirus was reported. On further study, this avirulent variant again yielded a virulent progeny, (designated as LH-v). These 2 variants served as testing tools and with them the effect of various factors on viral virulence was studied and the results are here reported.

Materials and methods. Tissue culture. Primary cultures of trypsinized monkey kidney cells were obtained from a commercial source,† or prepared according to the method described by Dulbecco and Vogt(2) and modified by Youngner(3). These cells were grown in 199 medium with 2% calf serum or in Melnick's medium(4) consisting of 0.5% lactalbumin hydrolysate in Hanks' balanced salt solution with 2% calf serum. After growing for about a week, when a complete sheet of cells was formed, the medium was poured off, the cells were washed and fresh medium was added. Such cultures 8 to 15 days old were ready for use in titration, neutralization or passage experiments.

Serial passage cultures of HeLa cells grown in Eagle's basal medium with 20% human serum were supplied commercially.†

All quantitation of the virus in the various harvests was carried out in monkey kidney tissue culture in roller tubes containing lactalbumin hydrolysate medium with 2% calf serum. Serial 1 log or half log dilutions of the virus were made in neutral Hanks' balanced salt solution and 5 or 6 tubes each containing 1.8 ml of medium, were inoculated with 0.2 ml of each of at least 4 dilutions. The tubes were incubated for 7 to 8 days. The tubes were observed microscopically every 2 or 3 days, and the ID_{50} was determined by the Reed and Muench method.

Media. Eagle's basal medium was prepared according to a recently modified formula(5,6). Medium 199 was purchased in

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