Glycerol Intake, Blood Cholesterol Level and Anemia in the Guinea Pig and Rabbit. (27875)

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A short report of Orekhovich and Plotnikov(1), indicating that the serum cholesterol level of rabbits which had received glycerol by stomach tube rose dramatically, prompted the following experiments. Ten guinea pigs and 4 rabbits received a 50% glycerol solution in saline or saline alone either by stomach tube or from a drinking cup daily for 30 to 40 days. The plasma cholesterol levels of all animals receiving glycerol, the red blood cell cholesterol levels of the rabbits and the red blood cell counts of the guinea pigs were compared with the respective values of the animals receiving saline only.

Results and discussion. Guinea pigs given more than 5 ml of a 50% glycerol solution daily by stomach tube died with acutely toxic symptoms. Rabbits tolerated 10 ml daily. The amount of glycerol solution consumed by the animals could not be established accurately. It very probably was more than that given by stomach tube. Those animals which were autopsied at the end of the experiment showed no gross pathological changes on cursory examination. In contrast to the above mentioned report(1), in neither species of animals did the level of the plasma cholesterol show consistent changes attributable to the intake of glycerol, nor did the red blood cell cholesterol levels of the rabbits show any consistent changes (Table I). The cholesterol levels of all animals, including those given saline only, fluctuated widely, especially during the first 2 weeks of the experiment. Variations with stage of estrous cycle cannot account for these variations since they were evident in males as well as in females. Likewise, comparison of water intake and plasma cholesterol values for the individual animals did not indicate that the variation was due to changes in blood volume. A typical example is shown in Fig. 1. To assess the possible effect of variations of degree of hemolysis on plasma cholesterol levels, the hemoglobin concentration of the rabbit plasma samples was estimated by the acid hematin method(3). The contribution of cell cholesterol to plasma cholesterol based on these measurements was in most cases less than 3%. In 2 samples showing considerable hemolysis high cholesterol values were also observed.

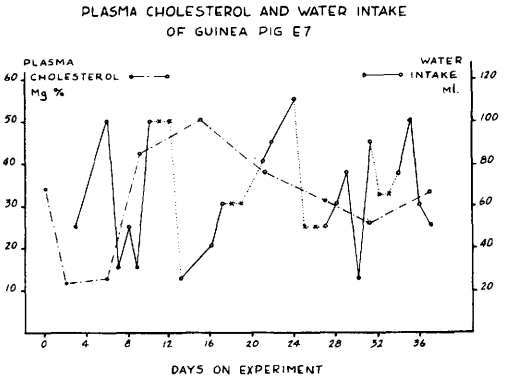

FIG. 1. Water intake figures indicated by × and dotted lines are avg of total intake over the 2 or 3 days over which it was measured.

The red cell count of guinea pigs receiving glycerol dropped during the experiment: Animals E4 6.2 to 3.0, E7 5.8 to 4.5 and C8 7.3 to 4.1 (times 10^6 cells per mm^3) respectively. Unfortunately, the saline-dosed animals were not really good controls. One of them had an unusually erratic drinking behavior which was reflected in fluctuations of the blood counts, while the other one had a relatively low blood count during all of the period of 20 days during which it was followed. In view of the fact that cholesterol feeding produces an anemia in guinea pigs and rabbits (4,5), it is interesting that glycerol feeding might be shown to produce a similar anemia without a rise in plasma cholesterol levels. The possibility that the observed anemia may be part of an unspecific effect of the glycerol should not however be overlooked.

Summary. A solution of either glycerol in saline or saline alone was administered

TABLE I. Blood Cholesterol Levels of Rabbits and Guinea Pigs Receiving Either Glycerol or Saline Solution.

Treatment	Animal No.	Days on experiment 0	2	8	15	32	55
Rabbits							
Plasma cholesterol (mg %)							
Glycerol, ST*	1	26.8	35.2†	23.8	33.8	24.4	—
" , "	2	28.2	—	—	42.6	30.4‡	—
" , drink	3	14.4	—	103.4§	34.8‖	31.8	20.0
Saline, ST	1	35.4	—	—	20.0	31.2	—
" , "	2	34.6	29.4	27.0	59.2	29.3	—
Cell cholesterol (mg %)							
Glycerol, ST	1	126	144	142	127	153	—
" , "	2	—	—	—	158	134	—
" , drink	3	122	—	150	—	—	124
Saline, ST	1	—	—	117	—	139	—
" , "	2	112	167	159	173	167	—

Treatment	No. & sex	Days on experiment 0	2	6	9	15	21	27	31	37
Guinea pigs										
Plasma cholesterol (mg %)										
Glycerol, ST	E4 ♂	21.8	12.0	13.6	38.7	15.0	28.0	28.6	24.9	24.5
" , "	E7 ♀	34.2	11.8	12.8	42.7	50.6	38.2	31.7	25.0	34.0
" , drink	C8 ♀	—	27.0	22.2	82.2	—	—	—	—	—
Saline, ST	C6 ♀	22.0	30.4	28.6	14.5	11.2	—	60.2	22.2	15.8
" , "	C1 ♀	32.7	—	13.8	—	27.8	29.0¶	38.3**	47.0	—
Heart puncture	C8 ♀	—	16.6	15.0	22.1	28.4	27.8	—	—	—

The rabbits were all males, weighing 2300-2500 g at beginning of experiment, 2700-2800 g after 50 to 60 days. They were given daily 10 ml of a 50% solution of glycerol in 0.9% sodium chloride or saline solution by stomach tube. Animals 1 and 2 were made to serve as their own controls: No. 1 was given glycerol for the first 38 days followed by saline for 23 days; No. 2 received saline first, followed by glycerol.

The guinea pigs maintained their weight of 500-730 g respectively during the experiment. They received 5 ml of the glycerol or saline solution respectively, daily.

All animals were given water and a commercial laboratory chow *ad lib.*

Blood samples were obtained by heart puncture from the guinea pigs, from an ear vein from the rabbits. No anaesthesia was used. 0.5 ml of plasma was extracted with 10 ml of ethanol-acetone (1:1) and cholesterol determined by the method of Sperry and Webb(2). Blood cells were washed 3 times with saline, hemolyzed with water and cholesterol extracted and determined as above.

Animal C8 served first as a control of the effects of repeated heart punctures on plasma cholesterol levels without administration of either glycerol or saline (23 days). This was followed by 13 days of drinking glycerol. At autopsy it was found to be pregnant.

* ST = stomach tube.
† Hemolysis approximately 15%.
‡ At 23 days.
§ Hemolysis approximately 30%.
‖ At 23 days.
¶ At 19 days.
** At 23 days.

by stomach tube to groups of guinea pigs and rabbits. Their plasma and cell cholesterol levels and their red blood cell count were compared over periods of 30 to 50 days. Guinea pigs given more than 5 ml of a 50% glycerol solution daily died with acutely toxic symptoms. Rabbits tolerated at least 10 ml daily. In neither species of animals did the level of the plasma or cell cholesterol show consistent changes attributable to the intake of glycerol. It appears probable that in the guinea pig the intake of glycerol is accompanied by an anemia.

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Distribution of Alpha Glyceryl Ethers in Animal Tissues.* (27876)

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Ethers of glycerol with long chain fatty alcohols were first discovered in 1922 by Tsujimoto and Toyama(1) in the non-saponifiable fraction of the liver oil of certain elasmobranchs. Progress on the chemistry and biological properties of these substances has been reviewed(2,3). Little is known of the distribution of the ethers in animal tissue. Karnovsky and co-workers(4) developed a quantitative method of analysis for the ethers based on measurement of formaldehyde liberated by periodate. This was applied to the non-saponifiable fraction from a variety of fish oils and tissues(5,6,7). Among land animal tissues the ethers have been found in intestine and liver of rats(5), in bovine yellow bone marrow(8), erythrocytes(9), brain(10) and butter fat(11), pig spleen(12), and human erythrocytes(13) and atherosclerotic arteries(14). Carter, Smith and Jones(15) isolated a glyceryl ether ethanolamine phosphatide from egg yolk which was resistant to alkaline hydrolysis. Inasmuch as several dog tissues contain appreciable amounts of alkali resistant ethanolamine lipids other than plasmalogen(16) it was of interest to determine if this fraction might be identified with the glyceryl ether phosphatide.

Method. Preparation of lipid hydrolysates. Tissue lipid extracts were prepared, purified and analyzed for phosphorus using methods described previously(16). The glyceryl ethers were liberated by the following hydrolysis procedure. A dried aliquot equivalent to about 0.3 mmole of lipid phosphorus was refluxed on a sand bath for 4 hours in 40 ml 1 N ethanolic KOH. 13.3 ml concentrated HCl were added and the mixture refluxed for another 12 hours. After cooling the alcohol was evaporated *in vacuo* and the hydrolysate extracted 3 times with a total of 50 ml chloroform. The chloroform extract was dried *in vacuo* and taken up in 20 ml petroleum ether.

Isolation of glyceryl ethers. The petroleum ether solution was then fractionated on a silicic acid column by the method of Hirsch and Ahrens(17). In this procedure neutral lipids are first eluted with petroleum ether and mixtures of petroleum ether and ethyl ether (Fractions 1 to 6 inclusive). The glyceryl ethers are then eluted with ethyl ether (Fraction 7) and finally the phosphatides are eluted with methanol (Fraction 8). Hydrolysis recovery experiments with standard samples of batyl alcohol added to synthetic mixtures and to whole lipid extracts are shown in Table I. The batyl alcohol was well recovered from the synthetic mixtures, less so from the natural lipid extracts. It was found only in Fraction 7, as observed by Hirsch and Ahrens. If very large amounts of batyl alcohol were used, small amounts were carried over into Fraction 8.

Hydrolysis recovery experiments with triacetylsphingosine alone or added to synthetic mixtures are shown in Table II. Sphingosine was completely recovered in Fraction 8 on the basis of the nitrogen(16) content of the fractions.

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