Results and comments. There were no differences in urinary excretion of steroids between the unmedicated sea level and high altitude groups (Table I). These results agree with the findings of San Martin *et al.*(2). The quantitative similarities in the 2 groups of urinary excretion of 17,21-dihydroxy-20-ketosteroids indicated that the yields of these catabolites were identical, without, however, giving information about relative rates of production and catabolism of cortisol and cortisone in the 2 groups. The amount of injected cortisol appearing in the urine in the following 24 hours, as Porter and Silber chromogens, was the same in high altitude and sea level groups (Table I). These results and the equivalence in percent of steroid conjugation (Table I) suggested that the catabolism was the same in both groups. The similar steroid excretions reflected, therefore, similar production of cortisol by the adrenal cortex (It is assumed here that the administered cortisol is catabolized in the same manner as the endogenous one). Dexamethasone decreased urinary excretion of steroid, but did not differentiate the sea level and high altitude groups (Table I). These findings showed that rates of production of cortisol and cortisone of both groups were identical when endogenous ACTH was inhibited; and, therefore that the similarities between both groups were not promoted by ACTH stimulation but rather depended upon the adrenal cortices themselves.

Summary. No differences were found in urinary excretion of steroids between sea level and high altitude natives. The data indicated further that the catabolism of cortisol was the same in both groups. Inhibition of endogenous ACTH by Dexamethasone did not affect urinary excretion of steroids differently in the 2 groups.

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Effect of Vitamin B_{12}, Unsaturated Fat and Hydrolyzed Glucosecycloacetate on Bile Acid Excretion in Experimental Hyperlipemia.* (26931)

M. C. NATH AND D. M. BRAHMANKAR
Dept. of Biochemistry, Nagpur University, Nagpur, India

Many workers have reported that cholesterol administered to animals is metabolized and eliminated mostly as bile acids(1-3). The effect of different food fats on elimination of the bile acids has also been studied. Haust and Beveridge(4) demonstrated an increased output of bile acids in the feces of human subjects receiving corn oil in the diet as compared to those receiving butter fat. Srikantia and Gopalan(5) have recently shown that fecal output of bile acids in human subjects receiving unsaturated fat is higher than in those receiving saturated fat. Recent work of Gordon *et al.*(6) supports the view that hypocholesterolemic effect of sunflower oil is associated with an increase in fecal output of bile acids. As the unsaturated fats are known to lower the higher cholesterol levels of the serum and tissues, it is reasonable to assume that unsaturated fat may lower serum

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cholesterol levels by promoting the fecal output of bile acids.

Nath and Saikia(7) have reported that high fat (saturated) given to rabbits for a long period, induces hypercholesterolemia as indicated by elevated cholesterol levels and C/P ratio whereas unsaturated fat (linseed oil) and the same level of mixed fat (saturated and unsaturated) were found to maintain lower ester cholesterol values and C/P ratio. Vit. B_{12}, essential fatty acids, inositol and aqueous extract of hydrolyzed glucose cycloacetoacetate (GCA) also checked the rise of cholesterol levels of plasma and tissues in experimental hyperlipemia(8).

The present work was, therefore, undertaken to study the effect of hydrolyzed GCA as well as Vit. B_{12} and unsaturated fat (groundnut oil) on excretion of fecal bile acids in animals with hyperlipemia induced by feeding high saturated fat diet.

Materials and methods. Thirty male albino rats weighing an average 125 g were divided into 5 equal groups. Animals of Group I were given basal diet consisting of sucrose 16%, casein 20%, groundnut oil 20%, whole meal wheat flour 39%, salt mixture(9) 5%. Group II received saturated fat diet containing same percentage of sucrose, casein, whole meal wheat flour and salt mixture as in basal diet, but groundnut oil (20%) was replaced by Dalda† (20%). Group III animals were given the same diet containing mixed fat 20% (10% Dalda + 10% groundnut oil) in place of 20% Dalda. Vitamin mixture consisting of Vit. B_1 .4 mg, Vit. B_2 .4 mg, folic acid .2 mg, pyridoxine .3 mg, niacin 3 mg, calcium pantothenate 2 mg, inositol 20 mg, and codliver oil (Vit. A & D) 10-15 drops were added/100 g of the diet. The animals were supplied with food and water *ad libitum*. Animals of Groups IV and V which received high saturated fat diet as did Group II, were injected subcutaneously with hydrolyzed product of GCA (120 mg) and 5 μg Vit. B_{12}‡ respectively/100 g body wt/day.

† Hydrogenated groundnut oil, Hindusthan Lever Product Private, Ltd.

‡ Macrabin, Glaxo Laboratories, India Private Ltd.

GCA was prepared according to the method of West(10) as modified by Nath *et al.* (11). Hydrolyzed GCA was prepared by hydrolyzing 1 g of GCA with 5 cc of 2N HCl for 20 minutes in a boiling water bath. The pH was then adjusted to 7.2 with 2N NaOH. The solution was filtered and filtrate repeatedly washed with ether. Aqueous layer was separated and traces of ether were removed. The experiment was continued for 8 weeks, after which the rats were caged separately for fecal collection. Feces were collected for 4 days and stored in cold.

Bile acids estimation. Since bile acids in feces are not in the conjugated form, the measurements here were made on free bile acids, *i.e.*, cholic, and dihydroxycholanic acids. The method for determination of these 2 bile acids was similar to that adopted by Haust and Beveridge(4) for measurement of cholic and dihydroxycholanic acids in feces.

Extraction of feces and column chromatography. Extraction procedure followed was similar to that of Carey and Watson(12) and final resulting mixtures were subjected to column partition chromatography as suggested by Mosbach *et al.*(13), using celite column which utilized 70% aq. acetic acid as the stationary phase and petroleum ether-isopropyl ether (IPE) mixture as moving phase. Elution was started with a mixture containing 40% IPE in petroleum ether and followed by gradual increase in IPE up to 60% in petroleum ether. IPE 40 and IPE 60 fractions were collected separately and evaporated to dryness and dissolved in 65% sulphuric acid (AR Grade) and heated at 60°C for 15 minutes. The 2 bile acids were determined spectrophotometrically according to the method of Mosbach *et al.*(14) by differential absorption at 320 mμ and 385 mμ for cholic and dihydroxycholanic (deoxy cholic and chenodeoxycholic acids, the 2 isomers) acids respectively with the Beckmann DU Quartz Spectrophotometer. Since IPE 40 and IPE 60 fractions behaved spectrophotometrically like dihydroxycholanic acid and cholic acid respectively, they were reported as dihydroxycholanic and cholic acids.

At the end of the feeding period, the ani-

TABLE I. Values of Bile Acid Excretion in Feces and Total Plasma Cholesterol of Rats from Different Groups, with Standard Deviation of Mean Values (6 Rats/Group).

Exp. groups		Cholic acid (mg/rat/24 hr)	Dihydroxycholanic acid (mg/rat/24 hr)	Total plasma cholesterol, mg %
I	Basal diet	.530 ± .023	.553 ± .014	121 ± 12
II	Saturated fat (hydrogenated) diet	.372 ± .028	.414 ± .034	205 ± 11
III	Mixed fat (hydrogenated fat + groundnut oil) diet	.536 ± .052	.543 ± .036	174 ± 9
IV	Hydrogenated fat diet and GCA(h) inj. (120 mg/100 g body wt)	.801 ± .036	.726 ± .057	138 ± 15
V	Hydrogenated fat diet and Vit. B_{12} inj. (5 μg/100 g body wt)	.760 ± .033	.648 ± .06	152 ± 14

mals were sacrificed and total cholesterol of plasma was estimated according to the method of Schoenheimer and Sperry(15) as modified by Mookerjea and Sadhu(16).

Results. Table I shows bile acid values (cholic and dihydroxycholanic acids) of the feces of the rats of different groups. Since total bile acids were not estimated, values of 2 bile acids obtained here do not account for the total fecal bile acids. Bergstrom and Norman(17) demonstrated the modification of cholic acid to compounds containing ketonic groups in the feces. But we obtained values for cholic and dihydroxycholanic acids in feces of rats, as Haust and Beveridge reported in human feces.

The animals receiving 20% hydrogenated fat excreted less bile acids than those receiving basal diet containing unsaturated fat and mixed fat containing 10% hydrogenated fat + 10% groundnut oil. Plasma cholesterol levels of saturated fat ingested animals are higher than those of animals receiving unsaturated fat and mixed fat. Hydrolyzed GCA and Vit. B_{12} when injected to the saturated fat ingested animals show pronounced effect on excretion of fecal bile acids. Plasma cholesterol levels of the animals of Groups IV and V injected with hydrolyzed GCA and Vit. B_{12} respectively, are also much lower than those receiving hydrogenated fat.

Discussion. From the above observations, it is noted that elimination of bile acids in feces is affected by the nature of the fat. The hydrogenated fat (20%) in absence of unsaturated fat appears to have increased plasma cholesterol levels through its diminishing effect on fecal elimination of bile acids. When equal quantities of saturated and unsaturated fats are given maintaining total dietary fat at 20%, bile acid excretion is increased, accompanied by fall in total plasma cholesterol level. This effect can be attributed to the unsaturated fat.

Gordon *et al.*(6) have reported an increase of fecal bile acid elimination with a concomitant fall in serum cholesterol levels when sunflower seed oil was substituted for coconut oil in the diet. Byers and Friedman(18) have also observed that bile obtained from the common bile duct was richer in cholic acid following the feeding of unsaturated fats than following saturated fats.

The observations of other workers, and our findings support the possibility that increased excretion of the end products of cholesterol as bile acids may be one of the possible mechanisms by which substances like Vit. B_{12}, hydrolyzed GCA and unsaturated fat bring about reduction in plasma cholesterol levels. The mechanism of inhibition of bile acid excretion in animals fed hydrogenated fat can be supported by the observation of Alfin-Slater *et al.*(19) that in the absence of essential fatty acids, there is marked increase in the deposition of cholesterol esters of fatty acid which are not available for normal metabolism.

Vit. B_{12} and hydrolyzed GCA which was found to contain dienol glucose by Nath and Bhattathiry(20) may, by stimulating the cholesterol metabolism, restore the normal metabolism of cholesterol which is hampered by saturated fat. Nath and Saikia(21) have suggested that hydrolyzed GCA and Vit. B_{12} through their property of increasing the con-

tent of methionine in the liver, aid the biosynthesis of choline which results in the increase of phospholipid, and thus may prevent the rise of C/P ratio and atherosclerosis. Besides this, hydrolyzed GCA and Vit. B_{12} may be helpful in cholesterol metabolism.

Summary. The effect of Vit. B_{12}, unsaturated fat and hydrolyzed glucose cycloacetoacetate (GCA) on total plasma cholesterol levels and on excretion of fecal bile acids in rats with hyperlipemia induced by feeding saturated fat has been studied. These substances have been found to increase the output of fecal bile acids with a concomitant fall in total plasma cholesterol levels, thus indicating their important role in increasing the metabolism of cholesterol in the living system through its conversion to bile acids. Hydrolyzed GCA was found to be most effective in this process.

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Sex Differences in Maximal Hematocrit Values in the Rat in Response to $CoCl_2$ Administration.* (26932)

RICHARD H. SWIGART AND LAWRENCE G. GOLDBERG†
(Introduced by William B. Atkinson)
Department of Anatomy, University of Louisville School of Medicine, Louisville, Ky.

The effect of gonadal hormones upon erythropoiesis has been studied in many different species and several facts are generally agreed upon. Hematocrit levels, erythrocyte counts and hemoglobin content: (A) are higher in the intact male than in the intact female; (B) fall in the male and rise in the female following castration; (C) rise in the castrated male following testosterone administration and fall in the castrated female following estrogen administration. Furthermore, Finkelstein, *et al.*(1) have shown that the male rat recovers from hemorrhagic anemia faster than the female and that androgen accelerates while estrogen delays the recovery time. More recently Mirand, *et al.*(2)

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