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## Effects of Conjugated Bile Acids on *in vivo* Cholesterol Metabolism in the Mouse.* (26529)

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Considerable data have accumulated on the effects of various bile acids on rates of cholesterol synthesis in different tissues(1,2). If one couples these data with the finding that certain bile acids are principal end products of cholesterol metabolism(3,4), it appears most probable that these substances are active in the control of certain phases of cholesterol metabolism. However, free bile acids—not the glycine and taurine conjugates—were employed in this work. It is, therefore, possible that some of the observed effects were due to exhaustion of the supply of amino acids used in conjugate formation; *viz.*, glycine, serine, and cysteine. A deficiency of any of these could have a serious effect on cholesterol metabolism and on metabolism in general. To check this possibility, the glycine and taurine conjugates of a number of bile acids were synthesized and their effects on cholesterol metabolism compared with those of the free bile acids.

*Methods.* The conjugates of cholic, deoxycholic, and hyodeoxycholic acid were synthesized according to the method of Norman(5), with some modification. The purity of the conjugates was checked by paper chromatography(6) and elementary analysis.

*Elementary Analysis of Bile Acid Conjugates*

| | | Calc. | Found |
|---|---|---|---|
| Glycocholic acid | N | 3.01 | 3.25 |
| Sodium Taurocholate | S | 5.96 | 5.70 |
| Glycodeoxycholic acid | N | 3.12 | 3.32 |
| Taurodeoxycholic " | S | 6.15 | 6.04 |
| Glycohyodeoxycholic acid | C | 69.45 | 70.0 |
| | H | 9.64 | 10.37 |
| | N | 3.12 | 3.07 |

One hundred female albino mice of the Webster strain, weighing 21-26 g, were placed on a cholesterol free diet(7) for a 2-week period. They were then divided into 10 equal groups and diets supplemented as shown in Table I. All diets were fed *ad lib.*

After 3 weeks each animal received an intraperitoneal injection of 5 $\mu$c sodium acetate-1-$C^{14}$. One hour later the animals were sacrificed and livers removed for analysis of cholesterol and cholesterol-x-$C^{14}$(4). Cholesterol isolated from tissues was purified through the dibromide(8).

*Results and discussion.* Effects of free bile acids and of bile acid conjugates, are, in most cases, qualitatively similar (Table I). The conjugates of cholic and deoxycholic acids raise liver cholesterol levels to a greater extent than the free bile acids, but they do not have this increased effect on acetate-1-$C^{14}$ incorporation rates. However, these rates were lowered one-sixth to one-tenth by free cholic and deoxycholic acid, so that any further lowering would be difficult to detect with any certainty because of high deviations.

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Extract of hog bile was supplied by Wilson Laboratories, Chicago, Ill.

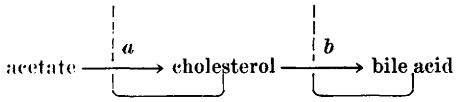
TABLE I. Effect of Bile Acids and Bile Acid Conjugates on Mouse Liver Cholesterol Levels and Synthesis Rates.

| | Dietary groups* | Total liver cholesterol, mg/g† | Cholesterol-x-$C^{14}$ activity, counts/min./g tissue |
|---|---|---|---|
| I | Controls | 5.32 ± 1.24 | 1176 ± 312 |
| II | Cholic acid | 6.73 ± 1.47 | 145 ± 64 |
| III | Glycocholic acid | 9.33 ± 1.61 | 241 ± 91 |
| IV | Sodium taurocholate | 8.52 ± 1.54 | 386 ± 205 |
| V | Deoxycholic acid | 4.52 ± 1.35 | 201 ± 80 |
| VI | Glycodeoxycholic acid | 8.41 ± 2.23 | 201 ± 109 |
| VII | Sodium Taurodeoxycholate | 7.86 ± 1.64 | 265 ± 142 |
| VIII | Hyodeoxycholic acid | 2.97 ± .39 | 3374 ± 909 |
| IX | Glycohyodeoxycholic acid | 3.28 ± .81 | 6827 ±1890 |
| X | Hog bile extract (45% glycohyodeoxycholic acid) | 3.41 ± .53 | 3286 ±1130 |

* Supplements were fed at level of 0.5% of diet.
† Wet wt.

The results for sodium taurocholate (Group IV) are not in accord with those reported by Siperstein(9), but his experimental conditions were different since he employed rats rather than mice. As previously pointed out(10), the effects of various bile acids exhibit a high degree of species specificity. Moreover, the effect of sodium taurocholate on lowering the rate of acetate-1-$C^{14}$ incorporation, atlhough pronounced, was significantly less than that of free cholic acid or its glycine conjugate.

Although cholesterol levels and synthesis rates are inversely related, this relationship is not quantitatively significant. For example, liver cholesterol levels in mice treated with hyodeoxycholic acid (Group VIII) and glycohyodeoxycholic acid (Group IX) are not significantly different; however, cholesterol synthesis rate for Group IX is double that for Group VIII. In another experiment previously unreported, liver cholesterol level of control mice was 7.25 mg/g while that of mice on a diet supplemented with 3% corn oil was 3.93 mg/g. This represents a 45% decrease in cholesterol level, but the cholesterol synthesis rate was the same in both groups. These facts are somewhat surprising if the double feedback mechanism of action of the bile




acids is valid. An increase in bile acid concentration blocks reaction sequence *b* causing cholesterol to accumulate. This blocks sequence *a*, resulting in diminished incorporation of acetate into cholesterol.

Siperstein(9) has recently found evidence that it is not cholesterol *per se* which inhibits its own synthesis, and this may explain the lack of a quantitative relationship. Furthermore, we may be approaching limiting conditions in these experiments.

Although the results show that the effects of bile acids are not mediated through exhaustion of the supply of certain amino acids, they do not show whether conjugated bile acids or free acids are responsible inasmuch as some hydrolysis of the conjugated acid by intestinal flora would be expected during hepato-intestinal recirculation.

Cholesterol-x-$C^{14}$ isolated from various groups was converted to the dibromide, regenerated and recrystallized. There was more or less loss of activity (Table II) in Groups I through VII. The activity of controls and of Groups VIII and IX changed only slightly. Thus the overall results are somewhat enhanced and the conclusions remain valid.

*Summary.* Free and conjugated bile acids had qualitatively similar effects on mouse liver cholesterol levels and *in vivo* cholesterol-x-$C^{14}$ synthesis rates. The effects of free bile

TABLE II. Specific Activity of Cholesterol before and after Dibromide Purification, Counts/Min./mg.

| | Dietary groups* | Before | After |
|---|---|---|---|
| I | Controls | 123 | 112 |
| II | Cholic acid | 23 | 17 |
| III | Glycocholic acid | 24 | 13 |
| IV | Sodium taurocholate | 51 | 43 |
| V | Deoxycholic acid | 78 | 73 |
| VI | Glycodeoxycholic acid | 30 | 19 |
| VII | Sodium taurodeoxycholate | 54 | 38 |
| VIII | Hyodeoxycholic acid | 245 | 240 |
| IX | Glycohyodeoxycholic acid | 89 | 88 |

* Supplements were fed at level of 0.5% of diet.

acids on cholesterol metabolism are not due to the exhaustion of amino acids used in formation of glycine and taurine conjugates.

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## A Method for Transplantation of the Rabbit Kidney.* (26530)

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The study of kidneys transferred from one dog to another has provided important information on the transplantation reaction. Hume(1) has recently reviewed the work of Carrel, Williamson, Dempster, Simonson and others in this field. However, extensive employment of the kidney in transplantation research has been limited because currently available surgical technics necessitate the use of large animals such as dogs. The expense of preparation, maintenance and sterile surgical procedures required in dogs has discouraged their widespread use in such studies. Some of these difficulties can be eliminated by transplanting organs in the rabbit. This report describes a method which for the first time makes possible chronic homologous transplantation of the rabbit kidney by vascular anastomosis. This method has been employed to show the histological changes in the rabbit kidney during the rejection process.

*Method. Selection and preparation of host.* The recipients of kidney transplants were randomly bred albino rabbits, weighing about 4.0 kg. They were anesthetized by intravenously administered Nembutal (25 mg/kg) and placed on their backs with legs securely tied to the operating table. In this position, the retraction of the rabbit's neck often produces difficulties with respiration. To overcome this problem, 100% oxygen was administered under the surgical drape which covers the animal's head. The drape acted as a tent which partially contained the gas.

An intravenous drip containing 30 mg Nembutal and 2.5 mg heparin per 100 cc saline was connected to a peripheral ear vein. The rabbit received about 75 cc of this solution during a 2½ hour period. The heparin was given during surgery because it appeared to help prevent clotting at sites of the anastomoses. No additional anticoagulant was given to the host after operation.

A clean surgical procedure was used, but sterile precautions were not observed. The carotid artery and jugular vein on one side of the recipient rabbit's neck were isolated, clamped and divided, then their distal ends

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