is significant at the 5 percent level. This agrees with findings of adrenal hyperactivity (*5–7, 13*) in crowded *Mus* populations and supports the conclusion that a stressed condition was induced in the crowded colonies.

The reproductive behavior of the crowded and isolated animals is summarized in Table 1. The results indicate that the crowded populations of deer mice show a decreased reproductive potential. Implantation scars showed that all but one of the crowded females became pregnant, while the number of scars, though somewhat smaller in the average member of this group, was not significantly reduced as it might have been under more extreme crowding.

Intra-uterine mortality was found in both the experimental and control animals, as indicated by the greater number of implantation scars than of developing embryos. There was, however, a much higher percentage of intra-uterine mortality in the crowded mice. The number of viable fetuses in the crowded group represents an effective reproduction rate only 40 percent of the rate for the uncrowded mice, and the difference (F ratio = 4.30) is significant at the 2 percent level of confidence.

It is therefore concluded that prenatal mortality is probably one of the means of lowering reproductive performance in dense populations of breeding mice. The inference that hormones of the adrenal cortex induce fetal resorption, though reasonable, requires further experimental proof, whereas ecologists will be interested in field confirmation of the suggestion that moderate crowding can act as a stressor in Selye's sense (*14*).

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Studies on the Regulation of Fatty Acid and Cholesterol Synthesis in Avian Liver

Abstract. Homogenates of pigeon liver have been incubated with acetate-C^{14} in excess. Simultaneously, substrate for glycolysis was provided as glucose-6-phosphate. The incorporation of carbon-14 into cholesterol was maximal at low levels of glycolysis, whereas fatty acid turnover was maximum at higher glycolytic levels. A regulatory mechanism is proposed to explain the differential synthesis of cholesterol and fatty acids.

An interesting problem in the control of metabolic pathways is presented by the differential synthesis of fat and cholesterol under various pathologic conditions. For example, diabetic liver can synthesize cholesterol, but cannot synthesize fat normally. Supplementation with large amounts of glucose or fructose in vivo improves the rate of incorporation of acetate into fat by diabetic liver (*1*).

The carbon substrate for the synthesis of both fat and cholesterol is acetyl coenzyme A (acetyl CoA), and synthesis of both requires a hydrogen source. In each case the hydrogen source probably derives from the glycolytic pathway (*2, 3*).

A simple, but perhaps not altogether trivial, hypothesis for the selective control of the pathway of incorporation of acetate into lipide is derived from an analysis of the over-all balance of the carbon and hydrogen requirements for the utilization of equivalent amounts of acetyl CoA for cholesterol or fat synthesis.

$$18\ \text{Acetyl CoA} + 8\ \text{H} + \text{HOH} \longrightarrow \text{cholesterol} + 9\ \text{CO}_2 + 18\ \text{CoA}^{-\text{SH}}$$

$$18\ \text{Acetyl CoA} + 64\ \text{H} \longrightarrow 2\ \text{stearic acid} + 18\ \text{CoA}^{-\text{SH}} + 14\ \text{HOH}$$

This balance shows that the net molar hydrogen requirement for fat synthesis is about eightfold greater than the hydrogen requirement for cholesterol synthesis. It may be assumed that the diabetic animal in ketosis has a plethora of acetyl CoA, so that the controlling substrate factor might be the other requisite, the hydrogen source. Restoring glycolysis by the administration of glucose in vivo enhances the incorporation of acetate into fat in experimental animals (*1*) and relieves ketosis in the human diabetic (*4*). We therefore propose that the selections of pathways for utilization of acetate depend upon the total available reduced coenzmye, which is a function of the rate of glycolysis. To test this hypothesis, homogenates of pigeon liver were prepared and incubated with acetate-1-C^{14} in excess. Substrate for glycolysis was provided as glucose 6-phosphate.

Ten-percent homogenates of pigeon liver in 0.1M potassium phosphate (pH 6.5) were dialyzed against ten volumes of this buffer at 4°C for 1 hour to unmask a dependence of acetate incorporation on added glycolytic substrate. Longer dialysis resulted in an inactivation of acetate incorporation which could not be restored by the addition of nucleotides or coenzyme A. Preincubation with beta-amylase to destroy glycogen is not required. The dialyzed homogenate was supplemented with diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN) (*5*), Mg^{++}, Mn^{++} (*6*), coenzyme A, and acetate-1-C^{14} in excess. Two milliliters of this suspension were added to tubes containing glucose-6-phosphate and $KHCO_3$ (*7*), and the resulting solution was made to a final volume of 3.0 ml with 0.15M KCL and incubated with vigorous shaking for 1 hour at 37°C. Two milliliters of saturated KOH were added, and the mixture was saponified at 100°C for 2 hours. Mixtures of fatty acid and cholesterol were isolated according to the method of Hotta *et al.* (*8*) and counted for radioactivity.

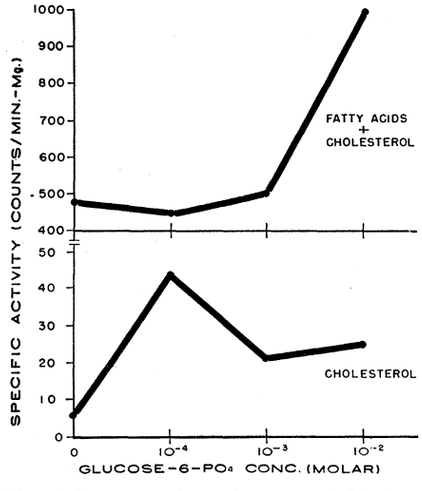

Fig. 1 Incorporation of acetate-1-C^{14} into fatty acids and cholesterol. Each tube contained $1.5 \times 10^{-4}M$ DPN, $1.5 \times 10^{-4}M$ TPN, $3.7 \times 10^{-3}M$ $MgSO_4$, $2.3 \times 10^{-4}M$ $MnCl_2$, $3.3 \times 10^{-3}M$ $KHCO_3$, 0.45 μg CoA, and $1.5 \times 10^{-3}M$ NaOAc-C^{14} (15 μc).

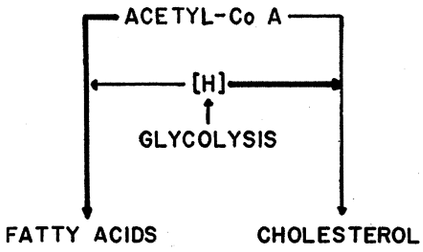

Fig. 2 Diagram of proposed regulatory mechanism to explain the differential synthesis of cholesterol and fatty acids.

Cholesterol was separated from the mixture as the digitonide (*9*) and counted. The resulting specific activities were corrected by zero time controls.

A typical experiment is shown in Fig. 1. Both cholesterol and fat incorporation were minimal in the dialyzed homogenate. Although incorporation into cholesterol is less than one-tenth of that into fatty acid, addition of small amounts of glycolytic substrate (glucose-6-phosphate) resulted in considerable enhancement of incorporation of C^{14} into cholesterol. As the concentration of glucose-6-phosphate was increased, C^{14} incorporation into total lipide increased, with concomitant diminution of incorporation into cholesterol. The availability of glycolytic substrate seemed to direct the anabolism of acetate to cholesterol or fat when the system was not limiting in acetate concentration.

The fact that the incorporation into cholesterol was optimal at low glucose-6-phosphate concentrations suggests a regulatory mechanism involving a limited supply of some component other than reducing equivalents. This is represented diagrammatically in Fig. 2. The data suggest that fat synthesis has a greater requirement for hydrogen equivalents than cholesterol synthesis. This is in agreement with the results of Siperstein and Fagan (*10*).

When acetyl CoA is generated at a constant rate it is mainly diverted toward fatty acid if an adequate hydrogen source (rapid glycolysis) is present, and only a relatively small amount finds its way into cholesterol. When, however, glycolysis is limiting, cholesterol synthesis is less affected than fatty acid synthesis, since it proceeds at a much slower rate and has a lower temporal as well as molal requirement for hydrogen. At certain levels of glycolysis (less than $10^{-4}M$ glucose-6-phosphate in Fig. 1) only fatty acid synthesis is diminished, and the decreased utilization of acetyl CoA by this system effectively diverts more acetate toward cholesterol, leading to the increased incorporation observed. Higher reducting potentials promote fatty acid synthesis which competitively withdraws acetyl CoA and thus decreased incorporation into cholesterol. Therefore, the net incorporation of acetate into total lipide is determined by the availability of both intermediate acetyl CoA and reduced coenzyme, whereas the partition of incorporation between fat and cholesterol is determined primarily by the availability of reducing potential.

The nature of the hydrogen source for fat synthesis has been elaborated by Green *et al.* (*11*), Langdon (*12*), and Siperstein and Fagan (*5, 10*), and appears to be a combination of the reduced forms of DPN and TPN. In our experiments glucose-6-phosphate furnished a source for reduction of both cofactors. The requirement of glycolysis for cholesterol synthesis has been demonstrated by Bucher and McGarrahan (*2*), but the exact cofactor requirement is still obscure. It appears from our experiments that the rate of glycolysis is a selective determinant for these two pathways, and it is suggested that the type of mechanism proposed above may warrant consideration as a general mode of differential biological regulation.

This interpretation suggests a close tie between nutrition and selective lipide synthesis. It is particularly interesting from the standpoint of the possibility that a localized enzymatic lesion in the arterial wall which results in diminished glycolysis might cause enhanced cholesterol synthesis and result in arterial cholesterosis (*13*).

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Biological Activity of 3-Methoxy-Catecholamines

Abstract. The methoxy analogs of epinephrine, norepinephrine, and dopamine manifest some activity on smooth muscle and blood pressure. Metanephrine is as potent as epinephrine on rabbit aortic strips. The same compounds demonstrate a lesser potency on rabbit duodenum preparation, while 3-methoxy-dopamine produces a slight contraction of the duodenum. Both metanephrine and 3-methoxy-dopamine have 15 to 25 percent of the pressor activity of the original nonmethylated catecholamines. Normetanephrine is 1/600 as active as norepinephrine on the blood pressure of the cat.

The discovery of O-methyltransferase (*1*) has been followed by a reassessment of the role of amine oxidase in the biological inactivation of catecholamines. Recently, many authors have indicated (*2*) that substances which inhibit O-methyltransferase are also potentiators of catecholamines. However, there have been only a few preliminary experiments (*3*) to indicate that 3-methoxy-catecholamines are biologically inactive substances.

In the present experiment (*4*) the 3-methoxy derivatives of epinephrine, norepinephrine, and dopamine were tested in vitro on rabbit aorta spirals, according to the method of Furchgott and Bhadrakom (*5*), and on rabbit duodenum (*6*). The results are shown in Table 1, and the activities of the methoxy derivatives are compared with those of epinephrine. The same compounds were also tested for their effect on the blood pressure of the anesthetized, atropinized cat. These results also are summarized in Table 1.

All of the 3-methoxy derivatives are capable of contracting the aorta spirals at various degrees. These compounds were far less active on rabbit duodenum, where metanephrine and normetanephrine are, respectively, 1/40 and 1/200 as potent as epinephrine. On the contrary, 3-methoxy-dopamine behaves in this preparation as a mild cholinergic substance.

Both metanephrine and 3-methoxy-dopamine show an important activity on the blood pressure response. In comparing doses necessary for an equal response, these substances were found to be one-fourth to one-fifth as active as the nonmethylated compound. The case is different with normetanephrine, since this compound is only 1/600 as active as norepinephrine.

The experiments reported indicate that the methoxy derivatives of catecholamines are not entirely devoid of biological activity. It would seem logical to believe that O-methyltransferase is not the only enzyme involved in the