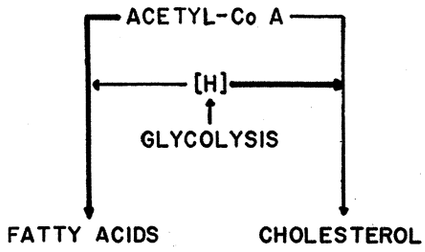

Fig. 2 Diagram of proposed regulatory mechanism to explain the differential synthesis of cholesterol and fatty acids.

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

Table 1. Comparative activity of 3-methoxy-catecholamines.

Rabbit smooth muscle preparation			Blood pressure response in cat
Aortic strip		Duodenum	
Threshold ($\times 10^{-7}$)	Potency*	Potency†	Response of nonmethylated analog (%)‡
		Epinephrine	
0.1	1.	1.	100
		Metanephrine	
3.	1.18	0.025	25.
		Normetanephrine	
2.3	0.17	.005	0.16
		3-Methoxydopamine	
19.	.04	Contraction	15.

* Ratio between concentrations of epinephrine and methoxy-catecholamines required for production of 50 percent maximum contraction. † Ratio between concentrations of epinephrine and methoxy-catecholamines required to produce the same degree of inhibition. ‡ This percentage was obtained by comparing the doses required to obtain an equal rise of blood pressure with the methylated and nonmethylated catecholamines.

biological inactivation of catecholamines or that this enzyme is not equally effective for the inactivation of all catecholamines.

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25 March 1960

Influence of Uterine Site on Occurrence of Spontaneous Cleft Lip in Mice

Abstract. It has been found that within the A/Jax strain, embryos in the uterine site nearest the ovary develop cleft lip (with or without cleft palate) significantly more often than embryos in other positions in the uterus.

When anomalies occur in an inbred strain of mice, why do they occur only in certain members of the litter and not in others? Litter mates in an inbred strain have very similar, if not identical, genetic constitutions and, furthermore, they are all subject to the same external and maternal environment. The differences that determine that one embryo is abnormal and that others are not must reside within the uterus, and since the differences are not obvious, they must be small, although probably not simple. This report is concerned with one such difference—namely, that of a difference in the sites occupied by embryos within the uterus and the influence of this difference on the occurrence of cleft lip in the highly inbred A/Jax strain of mice. Cleft lip with or without cleft palate has been reported as occurring with a frequency of 6.33 percent in A sublines related to the A/Jax strain (*1*).

The uterus was removed from each of 76 A/Jax females 14 or 15 days after insemination and, after fixation, the positions of embryos with spontaneous cleft lip were noted. A total of 485 embryos from uterine horns containing two or more embryos had an over-all frequency of cleft lip of 8.5 percent. There were no differences in frequency between right and left horns. The frequency of cleft lip at the ovarian site ($17/126 = 13.5$ percent) is significantly higher ($p < .02$) than the total at all other positions ($24/359 = 6.7$ percent). This tendency for cleft lip to occur more often in the embryo occupying the site nearest the ovary appears even more clearly when only those horns (35 horns in 29 litters) that contain at least one affected embryo are considered. Again the proportion of embryos with cleft lip is significantly higher ($p < .01$) for embryos nearest the ovary ($17/35$) than for embryos at other positions ($24/116$) in the uterine horn. In Table 1 the data are arranged according to the number of embryos in a horn. It can be seen that there is a tendency for embryos with cleft lip to occur more frequently at the site nearest the ovary regardless of the number of embryos within a horn, but that this tendency appears to be greatest when there are either few or many embryos in the horn.

Further support for the foregoing observations was obtained from another group of A/Jax mice primarily intended for another purpose. In this group 48 litters were collected, just before term, from mothers that had received cortisone 11, 12, and 13 days after insemination. In this group of cortisone-treated fetuses (see Table 1) the tendency for cleft lip to occur more often at the ovarian site is again seen, but the difference in over-all frequencies ($8/76$ versus $8/161$) is not significant. However, when only horns containing at least one affected embryo are considered, there are significantly more embryos with cleft lip ($8/15$) at the ovarian site than at the other sites ($8/35$); p, determined by Fisher's exact method, equals .03.

Table 1. Number and percentage (in parentheses) of cleft-lip (CL) embryos at the site nearest the ovary and at other uterine sites; the data are grouped according to the number of embryos per horn (horns containing one embryo are not included).

Embryos in horn (No.)	CL embryos (No. of total)			
	Ovarian site		Other sites	
	Untreated group			
2	4/26	(15.4)	1/25	(4)
3	5/32	(15.6)	2/64	(3.1)
4	2/31	(6.5)	5/96	(5.2)
5	3/22	(13.6)	6/89	(6.7)
6	1/8		3/40	
7	2/4	(20.0)	4/24	(11.8)
8	0/3		3/21	
Totals	17/126	(13.5)	24/359	(6.7)
	Cortisone-treated group			
2	4/31	(13.0)	1/31	(3.2)
3	1/18	(5.6)	2/36	(5.6)
4	2/16	(9.4)	4/48	(8.3)
5	0/9	(9.1)	1/36	(2.2)
6	1/2		0/10	
Totals	8/76	(10.6)	8/161	(5.0)

The data show clearly that cleft lip is more likely to occur in an A/Jax embryo that occupies the uterine site next to the ovary than in an embryo at any other site. At present it is not known what there is about this part of the uterus that makes it less favorable to normal development than the other parts. Perhaps there is a reduced blood supply which tips the balance, in a developmentally unstable embryo, in favor of abnormal rather than normal development. Since the ovarian site appears to be less favorable whether there are many or few embryos in the horn, the difference is more likely to be an inherent quality of that area of the uterus than an effect of other embryos in the horn competing for nutrients. The idea that this part of the mouse uterus is less favorable to the normal development of the embryo is supported by the work of Hashima (*2*), who found that embryos near term occupying the site nearest the ovary weighed significantly less than those at other uterine sites. It has also been noted by McLaren (*3*) that there is a tendency for "runts" in the TO random-bred strain of mice to occur significantly more often at the ovarian site than at other sites in the uterus. Gross examination of the blood supply, in vivo india-ink injections, and attempts to reduce the blood supply to individual embryos or to the entire uterine horn have not yet led to any significant findings (*4*).

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