which gave rise to it, is high (*3*). On the other hand, a general classification can never be perfect for all purposes. As emphasized by Sokal and Sneath (*4*), when we put together entities with the highest proportion of shared attributes, we debar ourselves from insisting that these entities share any one particular attribute. Thus a special classification is demonstrably the best one for the limited purpose for which it was constructed, a general one the best for a wide range of potential purposes.

Viewing the problem in this light, we can readily comprehend the distinction between our usual Linnaean system of classification and any particular folk system of classification. The former, by continual review, is consciously made more and more general (*4, 5*); the latter, perhaps unconsciously, is made more and more special—hence specific—and with the highest possible predictive value with respect to the operations for which it is employed. It is hardly surprising that the special classification will often be concerned with characteristics that are also reflected in the general one and, insofar as this is true, mirror it. This clearly tells us nothing about the structure of nature itself, but a great deal about our own view of this structure.

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Norepinephrine Methylation in Fetal Rat Adrenals

Abstract. *The activity of phenylethanolamine-*N*-methyl transferase, the enzyme that methylates norepinephrine to form epinephrine, increases rapidly in the fetal rat adrenal during the day preceding epinephrine accumulation. The developmental increase in enzyme activity and the accumulation of epinephrine are prevented by fetal hypophysectomy (decapitation). Administration of adrenocorticotrophic hormone or cortisol acetate largely reverses the effect of fetal decapitation.*

The probable regulation by the adrenal cortex of adrenal medullary production of epinephrine in the adult, first proposed 15 years ago (*1*), has long been studied in rat and rabbit fetuses (*2*) in our laboratory. Concurrent with the initiation of adrenocortical function, there is a rapid accumulation of epinephrine in rat and rabbit fetal adrenals (*3*). Moreover, an intact hypophyseal-adrenocortical system is required for normal levels of epinephrine to accumulate in the fetal adrenal (*4*). Thus fetuses deprived of their hypophyses by decapitation, prior to the onset of accumulation of epinephrine, manifest at term only about one-fourth of the normal adrenal content of epinephrine. By contrast, norepinephrine accumulates to above-normal levels following fetal decapitation, although the total content of epinephrine-plus-norepinephrine diminishes almost to half of normal. This effect can be obviated if at the time of operation one injects either adrenocorticotrophic hormone (ACTH) or cortisol acetate into the fetus (Table 1). These results prompted our investigation (*5*) of the enzyme phenylethanolamine-*N*-methyl-transferase (PNMT), which methylates norepinephrine to epinephrine in the adrenal medulla (*6*), and of its regulation by the fetal hypophyseal adrenocortical axis. Similar studies of the adult rat have been reported (*7*).

Pregnant female rats of the Sherman strain were killed by cervical fracture at various times post coitum, and adrenals from fetuses of the same age were pooled for the determination of PNMT activity (*7, 8*). To study the effect of the fetal hypophysis on PNMT activity, fetuses were deprived of their hypophyses by decapitation *in utero* 17.5 days post coitum (*9*). At 19.5 days these pregnant females were reoperated for administration of ACTH (1 unit in olive oil) or cortisol acetate (0.5 to 1.0 mg in 0.9-percent NaCl) intraperitoneally to the decapitated fetuses. In several instances females containing decapitated fetuses received subcutaneous injections of cortisol acetate (25 mg twice daily from day 17 to day 20 of gestation). At term (21.5 days), females were similarly killed, and extracts of fetal adrenals were prepared for PNMT determination or catecholamine assay (*10*); untreated littermates from operated (uninjected) females served as controls.

Our results indicate that the fetal adrenal gland at 17.5 days contains slight but significant PNMT activity, as well as traces of epinephrine (Fig. 1). The enzyme activity increases eightfold between days 17.5 and 18.5 while the epinephrine content doubles; thus the initial rate of increase of enzyme activity is faster than the rate of accumulation of epinephrine. Thereafter, epinephrine accumulation and PNMT activity increase rapidly.

The possibility that the increase in PNMT activity in the fetal rat adrenal during gestation was caused by the appearance of an activator or by the disappearance of an inhibitor, rather than by an increase in enzyme protein content, was con-

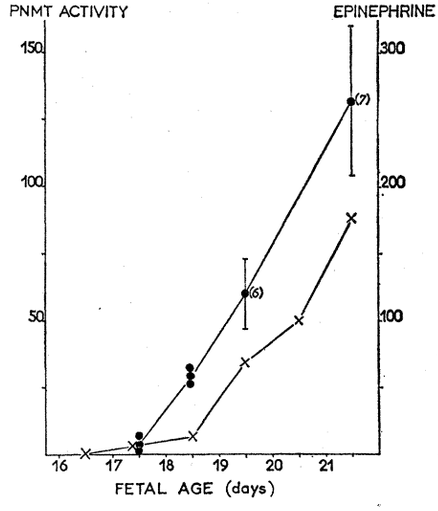

Fig. 1. Activity of PNMT (solid circles; micromicromoles per hour) and content of epinephrine (*3*) (crosses; nanograms) in pairs of fetal rat adrenals during gestation. Numbers of independent determinations are in parentheses; confidence intervals calculated for P .05.

Table 1. Effects of fetal decapitation and injection of ACTH or cortisol acetate (CA) on the activity of phenylethanolamine *N*-methyl-transferase (PNMT) on the epinephrine and norepinephrine contents of pairs of fetal rat adrenals. Values (means) are followed by ranges (in parentheses) and numbers of determinations (in square brackets); each pair of epinephrine and norepinephrine determinations was made from the one sample.

Treatment	PNMT ($\mu\mu$mole/hr)	Epinephrine (ng)	Norepinephrine (ng)
None	82.5 (69.7–114) [11]	208 (161–269) [11]	47 (31–71) [11]
Decap.	9.6 (3.5–16.3) [3]	51 (10–105) [13]	108 (30–185) [13]
Decap. + ACTH	63.4 (59.1–71.8) [4]	178 (149–209) [5]	60 (25–88) [5]
Decap. + CA	31.6 (22.6–43.4) [3]	202 (143–255) [5]	89 (61–120) [5]
Decap. in female receiving CA	32.9 (27.2–51.0) [7]		

sidered. Mixtures of extracts from 17.5-day and 21.5-day fetal adrenals manifested PNMT activities equaling the sums of the activities of the two; this fact is compatible with the idea of increased enzyme protein content during development.

Fetal decapitation reduces PNMT activity at term to only about 10 percent of that in littermate controls (Table 1). This reduction can be almost eliminated if, 2 days before they are killed, the pregnant females are reoperated and ACTH is administered to the decapitated fetuses; this treatment strongly stimulates the fetal adrenal cortex (*11*). When cortisol acetate rather than ACTH is administered to either the decapitated fetuses or the pregnant female herself, the activity of PNMT in the fetal adrenal is elevated to three times the untreated-decapitated level.

The PNMT activity does not reach the levels of controls after cortisol acetate treatment, although the adrenal content of epinephrine does seem to achieve control levels. Although the contents of the gland may not reflect the level of synthesis, but rather the result of the synthesis-release mechanism, this result suggests that in control fetuses PNMT is present in excess and that only one-third of normal enzyme activity would suffice to restore epinephrine content to normal.

One should now note what appears to be a manifestation of differential target organ sensitivity to glucocorticoids. In order to demonstrate a pronounced effect of fetal hypophysectomy or adrenalectomy upon fetal liver glycogen deposition, one must first perform maternal adrenalectomies (*2*, *12*) to reduce the circulating corticoids to negligible levels. We now report a profound effect of fetal hypophysectomy upon epinephrine content and PNMT activity in the fetal adrenal without performance of maternal adrenalectomy. Since the liver depends on corticoid levels in peripheral blood, while the adrenal medulla is irrigated mainly by blood deriving from the adrenal cortex and containing the highest corticoid concentration in the body, one might predict that the fetal liver is more sensitive to small fluctuations in corticoid levels than is the fetal adrenal medulla. Our results are compatible with such an explanation.

The discrepancy between the effects of injected ACTH and cortisol acetate in elevating the PNMT activity in the fetal adrenal may perhaps be similarly explained, since one may expect a higher local concentration of active corticoids to reach the fetal adrenal medulla following injection of ACTH than following administration of cortisol acetate.

Our data suggest that PNMT is not the sole entity responsible for the regulation of epinephrine accumulation, but is rather part of a series of events. Thus there is an apparent lag between the rise in PNMT activity at 17.5 to 18.5 days and the subsequent rise in the rate of epinephrine accumulation in the fetal adrenal gland (Fig. 1). During this interval the rate of synthesis of norepinephrine must increase to provide substrate for PNMT; thus, perhaps, it is this rate, rather than the PNMT activity, that becomes limiting. Moreover, although the amounts of epinephrine and norepinephrine are relatively reversed after decapitation, the total quantity of these two amines falls from 255 to 159 ng per pair of adrenals (Table 1). Thus not only has PNMT activity decreased, but, barring any change in secretory function, the rate of norepinephrine production as well; otherwise one would expect the total accumulation of these two amines following decapitation to equal the content in the controls.

Of the factors implicated, tyrosine, the first substrate in the catecholamine biosynthetic pathway, seems not to be rate-limiting. We have observed that the acid-soluble tyrosine content (*13*) of the whole body of the fetal rat remains at about 50 μg per gram of fetus ($3 \times 10^{-4}M$) from 15.5 to 19.5 days of gestation; this value is at least ten times the Michaelis-Menten constant for the enzyme tyrosine hydroxylase, the rate-limiting enzyme in the pathway between tyrosine and norepinephrine in the adult (*14*).

It is apparent from our results that the level of PNMT activity is of great importance in the initial development accumulation of epinephrine in the fetal rat adrenal, and further, that the level of this enzyme activity is controlled by the hypophyseal-adrenocortical axis. But more work is required to ascertain whether the changes observed in PNMT activity following decapitation, or administration of ACTH or cortisol acetate, are due to the regulation of PNMT synthesis or to some more general effect on the development of the adreno-medullary gland.

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