

Die von uns benutzte Modifikation der Inaktivierungs-Inhibitions-Methode ist mit der elektrophoretischen Trennung der Isoenzyme der alkalischen Phosphatase gut vergleichbar und ihre Resultate waren mit dem Knochen- und Leberfunktionszustand ( $^{85}\text{Sr}$ -Test und  $\gamma$ -Glutamyl-transpeptidaseaktivität) korreliert<sup>3</sup>. Dieselbe Methodik wurde früher für die Ermittlung von Referenzwerten bei Erwachsenen (20–60 Jahren) und älteren Menschen (61–76 Jahre) benutzt. Im Vergleich dieser Werte ist eine Altersabhängigkeit gut nachweisbar.

Der von uns gefundene Geschlechtsunterschied und Verteilungstyp der Gesamtaktivität, als auch die Blutgruppenabhängigkeit der Darmphosphataseaktivität im Serum stimmen mit den Ergebnissen anderer Autoren überein<sup>7–9</sup>. Auffällig sind die hohen Knochenphosphatasewerte, die vermutlich den Abschluss der Wachstumsphase bei den Studenten widerspiegeln. Daraus resultiert wahrscheinlich die log-normale Verteilung der Knochenphosphatase und – da die Knochenphosphatase den Hauptanteil an der Gesamtaktivität stellt – die der Gesamtaktivität. Die log-normale Verteilung der Darm- und Leberphosphatase könnte auf Ernährungseinflüsse zurückzuführen sein.

Die Abhängigkeit der Enzymaktivität von der Qualität und Quantität der zugeführten Eiweiße ist für einige Enzyme nachgewiesen worden<sup>10,11</sup>, diese Befunde lassen sich aus den Stoffwechselprozessen erklären. Die Aufgabe der Darmphosphatase in den Absorptionsprozessen und ihr Ansteigen im Serum nach fettreicher Ernährung sind gut bekannt<sup>9,11,12</sup>. Ein Ansteigen der Darmphosphatase im Serum fanden wir auch bei eiweissreicher Ernährung, ohne den Fettanteil der Nahrung streng zu kontrollieren. Beide Ernährungseinflüsse auf die Darmphosphatase sind in Tierexperimenten nachgewiesen worden<sup>13</sup>.

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## Changes in Dopamine $\beta$ -Hydroxylase Activity of Monkey Plasma with Age

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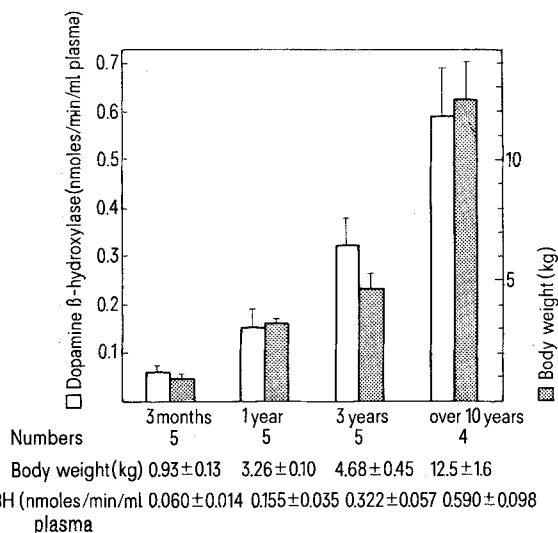
**Summary.** Plasma dopamine  $\beta$ -hydroxylase activity of Japanese monkeys (*Macaca fuscata fuscata*) increased with age, and the developmental changes were similar to those of human beings. However, the adult level plasma DBH activity of various monkey species was much lower than that of human beings.

Dopamine  $\beta$ -hydroxylase (DBH) activity in human blood (activity per ml serum or plasma) is relatively high, compared with that in other animals<sup>3,4</sup>, and increases with age for the first 2 or 3 years of life to a constant adult level<sup>5,6</sup>. Rat blood DBH activity per ml serum also increases after birth, but reaches a peak at 2–3 weeks of age, then decreases up to about 10 weeks to a constant

adult level (about 1% of human level)<sup>7,8</sup>. The rapid rise in development and relatively high adult level of DBH activity in human blood is considered to be attributed to the erect posture in human beings.

We have examined developmental changes in DBH activity of monkey blood in order to examine whether or not the developmental changes in human beings are similar to those in other primates.

Plasma samples were obtained by venipuncture with heparin as anticoagulant. DBH activity was determined by sensitive dual-wave-length spectrophotometry based on the spectrophotometric method of NAGATSU and UDENFRIEND<sup>4</sup>, as described by KATO et al.<sup>9</sup>. The incubation mixture (total volume 1.0 ml) contained: 400  $\mu\text{l}$  of 4-fold diluted plasma, 200  $\mu\text{l}$  of sodium acetate buffer, pH 5.0, 10  $\mu\text{l}$  of *N*-ethylmaleimide, 1 nmol of



Developmental changes of plasma dopamine  $\beta$ -hydroxylase activity in Japanese monkeys (*Macaca fuscata fuscata*). Values are expressed in mean  $\pm$  SEM.

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CuSO<sub>4</sub>, 10 μmol of sodium fumarate, 10 μmol of ascorbic acid, 1 μmol of pargyline, 1500 units (50 μg) of catalase, and 20 μmol of tyramine hydrochloride. Incubation was carried out at 37°C for 45 min. A sample of diluted plasma boiled at 95°C for 5 min was used for blank incubation. Changes in serum DBH activity during development of Japanese monkeys (*Macaca fuscata fuscata*) are shown in the Figure. Plasma DBH activity in young Japanese monkeys at 3 months after birth was very low, but increased gradually during development to the adult level (0.59 nmol/min/ml plasma), and the developmental changes were parallel to those of body weight. These developmental changes in monkey plasma DBH activity are similar to those in human beings.

Plasma DBH activity in various species of adult monkeys, i.e., chimpanzee (*Pan troglodytes*), hamadriad baboon (*Papio hamadryas*), and thick-tailed galago (*Galago crassicaudatus*) were: 0.05, 0.05 and 0.08 nmol/min/ml plasma, respectively. Therefore, the activities are different among various monkey species regardless of the difference in their posture, and distinctly lower than those of human beings (mean value, 43 nmol/min/ml serum or plasma<sup>4</sup>). It is concluded that although developmental changes in plasma DBH activity of monkeys are similar to those of human beings, the enzyme activity in plasma of various species of adult monkeys is much lower than that of human beings.

Cholesterol Esterification Activities in the Intestines and Pancreas of the Albino Rat

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Summary. Cholesterol esterification activities in intestines and pancreas are much greater with unsaturated fatty acids than with the saturated ones; the maximum activity is with arachidonic acid in intestines and with oleic acid in pancreas. The pancreatic cholesterol esterification activity is higher than the intestinal one.

In biological systems, there are at least three different mechanisms for the cholesterol esterification activities. The esterification reaction taking place in incubated serum is due to a transesterase which does not require any cofactor for its activity<sup>1-3</sup>. This enzyme has a specificity for fatty acids characteristic of the normal pattern of cholesterol esters. The liver is suggested as the source of the plasma enzymes. Contrary to the above mechanism, cholesterol esterification in the liver involves the presence of CoA, ATP, etc. as cofactors<sup>4,5</sup>. This enzyme has been shown to be a fatty acyl-CoA-cholesterol acyl transferase. In a third mechanism, the apparent direct reaction of free cholesterol with free fatty acid seems to be responsible for cholesterol ester synthesis in both the pancreas and the intestines<sup>6,7</sup>. This reaction has been studied with highly purified enzyme systems and certainly does not proceed through the formation of a fatty acyl CoA intermediate. In the present paper, the effects of two dietary fats on cholesterol esterification activities in the intestines and pancreas are discussed.

Materials and methods. 8 groups of 5 weanling male albino rats were fed safflower seed oil (highly unsaturated vegetable oil) or lard (animal fat) at 10, 20, 30 and 50% of their total calorie intake. The total calorie intake was kept constant. The rats were sacrificed at the end of an 8-week experimental period and cholesterol esterification activities in the intestines and pancreas determined by the method of MURTHY et al.<sup>7</sup>. Intestines and pancreas were cut and washed with ice-cold saline to remove food particles. They were pressed on filter paper and placed in -15° acetone. Acetone-dried powder was prepared by grinding the tissues in 10 volumes of acetone precooled to -15°C. The powder was suspended in distilled water (1 g/10 ml) for 1 h and centrifuged at 10,000 g for 10 min (0-5°C) and the clear supernatant was used as the enzyme source. Intestinal and pancreatic enzyme assays were carried out at 37°C in a metabolic shaker for 1 h and 30 min respectively. Reactions were terminated by adding 5 ml ethanol to the system and total and free cholesterol determined before and after the incubation period<sup>8</sup>. Protein was determined by the method of GORNALL et al.<sup>9</sup>.

Results and discussion. It is clear (Table I) that the process of esterification in intestines is much greater in the presence of unsaturated fatty acids than of saturated ones. The enzyme activity is the same when incubated

Table 1. Cholesterol esterification activity in rat intestines

Group	Palmitic	Stearic	Oleic	Linoleic	Arachidonic
10% Safflower oil	6.8	6.6	48.5	61.6	81.2
20% Safflower oil	7.8	7.8	41.4	69.3	85.1
30% Safflower oil	9.8	10.4	46.0	72.0	86.8
50% Safflower oil	8.0	9.0	50.4	70.4	84.8
10% Lard	10.0	9.4	53.3	67.4	86.0
20% Lard	10.0	9.4	50.0	66.0	86.4
30% Lard	12.4	12.4	52.0	70.8	88.0
50% Lard	10.1	10.7	49.6	68.5	83.5

The values are expressed as nmoles of cholesterol esterified/mg protein/h. The enzyme assay system contained in a total volume of 5 ml: 25 mg of intestinal powder corresponding to 8 mg protein, potassium phosphate buffer 0.1 M, pH 6.1, 3 ml, sodium taurocholate 25 mg, cholesterol 5 μmoles and fatty acid 10 μmoles.

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