This merger could not be ascribed to similar appearance, for there was no difficulty in recording other identical looking pairs who exhibited some clear-cut behavioral differences.

Consistent behavioral differences within some identical pairs deserves special attention. In pair E, following normal births, only the second-born was startled by noises in the first two months, cried at the jack-in-the-box at 3 months, and became extremely fearful of strangers during the last half of the first year. In pair K, the second-born twin, after a traumatic breech birth, slept much of the time over the first 1½ months. Then he began smiling to people more readily than did his brother whose delivery had been normal, and at 5 months he wanted to be picked up by any newcomer. Thereafter he remained more immediately outgoing to people than his twin. Our observations and interviews with the parents suggested that differential treatment played no role in producing these differences, and obstetrical and pediatric records yielded no clues. The following categories were examined, and none could be reasonably associated with such differences: birth order, traumatic delivery, large differences in birth weight, Apgar ratings (an assessment of viability at birth), and monochorionic versus dichorionic embryogenesis.

Although motor behavior was most effective in differentiating within twin-pairs, perhaps because it was most objectively scored, we cannot estimate heritability for specific aspects of behavior, nor was this our aim. Our question was rather: Does heredity, in a general sense, play a role in the development of abilities and personality? An affirmative answer would appear to be warranted (*6*).

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Cholesterol in Higher Plants

Abstract. *The presence of cholesterol in* Solanum tuberosum *and* Dioscorea spiculiflora *plants was demonstrated by gas-liquid chromatography, thin-layer chromatography, isolation and mixed melting point, and purification to constant radioactivity after dilution with authentic cholesterol.*

Although cholesterol, the principal animal sterol, has been isolated from red algae (*1*), its presence in higher plants has not previously been reported. In the course of our work on the biosynthesis of steroids in plants (*2*) we have identified this sterol in the sterol fraction of both *Solanum tuberosum* and *Dioscorea spiculiflora*.

Four young potato plants, *Solanum tuberosum*, of the Katahdin variety (*3*), were fed 25 μc each of mevalonic acid-2-C^{14} by the wick method (*4*) and allowed to grow for 1 month. The stems and leaves were homogenized with water in a Waring blender, hydrolyzed with 4*N* HCl, and filtered; the filter cake was washed with water to neutrality. This residue was extracted with light petroleum in a Soxhlet apparatus.

The extract (4.987 g) was chromatographed on neutral alumina (grade III), and the fraction was eluted with 50 percent benzene-light petroleum and again chromatographed. This gave 213 mg of crude sterols, which were acetylated and chromatographed on alumina. Gas-liquid chromatography (*5*) and thin-layer chromatography (TLC) on Anasil B (*6*), developed four times with a mixture of hexane and ether (96:4) indicated that this fraction contained a mixture of three sterol acetates with properties similar to the authentic acetates of stigmasterol, β-sitosterol, and cholesterol. A 53-mg sample of this mixture was chromatographed on 30-g columns of Anasil S by gradient elution (1 liter of 1 percent ether in hexane with increasing amounts of 20 percent ether in hexane) (*7*). Partial resolution of this mixture was obtained in the first 300 ml, which was rechromatographed. The middle fractions were enriched in a component with the mobility of cholesterol acetate, and the mixture was further purified by preparative TLC. Finally, a fraction was obtained which gave a single spot in thin-layer chromatograms on Anasil B. This fraction had the same mobility as an authentic sample of cholesterol acetate.

Gas-liquid chromatography of this fraction showed it to be a 1:1 mixture of two compounds corresponding in their retention times to cholesterol acetate and sitosterol acetate, respectively, although the latter had been completely removed by preparative TLC. When attempts to resolve the mixture by TLC failed, 2.2 mg of this mixture were subjected to preparative gas-liquid chromatography (*8*).

The fraction with the retention time of cholesterol acetate (200 μg) was recrystallized from 0.1 ml of methyl alcohol at 5°C and gave crystals melting at 112° to 116.5°C. The melting point of a mixture of this material with pure cholesterol acetate (mp 116.5° to 117.5°) was 114° to 117°C.

The crystals were then combined with the mother liquor, diluted with 26.3 mg of cholesterol acetate, and hydrolyzed to cholesterol. This material, having a radioactivity of 11,400 count/min per millimole, was purified through the dibromide (*9*). The radioactivity dropped to 8940 count/min per millimole and was not further changed by repetition of the dibromide purification step or by recrystallization from a mixture of ethanol and water.

Six young *Dioscorea spiculiflora* plants were fed 25 μc each of mevalonic acid-2-C^{14} through the stems for 30 days by the wick technique. The tubers and the upper portions of the plants were worked up separately as follows. After homogenization with water, the insoluble material was filtered off and extracted with petroleum ether and then with ethanol in a Soxhlet apparatus. The ethanol extract was combined with the filtrate and hydrolyzed

with 2*N* HCl; the neutral lipid fraction was obtained by extraction with benzene. The sterol acetates were isolated by preparative TLC of the lipid extracts, acetylation, and further preparative TLC. The fraction from the vegetative portions, 1.5 mg, had radioactivity of 174,000 count/min while the radioactivity of the tuber fraction, weighing 8.1 mg, was 109,000 count/min.

Both fractions gave three spots by TLC on Anasil B. The upper and lower spots had the same mobilities as β-sitosterol acetate and stigmasterol acetate, respectively, while the middle, much fainter spot corresponded to cholesterol acetate. Gas liquid chromatography gave three peaks, which corresponded to these three sterol acetates. Attempts to dilute the sterol acetate fractions with cholesterol acetate and then to purify to constant radioactivity showed that recrystallization does not remove the other sterol acetates and even purification by the dibromide is inefficient in this respect. Therefore the two sterol acetate fractions were combined, and by preparative TLC on Anasil B most of the β-sitosterol and stigmasterol acetates was separated so that a middle fraction enriched in cholesterol acetate remained. Reversed-phase TLC (paraffin oil and ethyl cellosolve-propanol-methanol-water, 45:8:25:22) on Kieselguhr G of a hydrolyzed portion of this fraction gave a spot having the same mobility as cholesterol and a lower spot corresponding to β-sitosterol and stigmasterol. To remove the rest of the other sterol acetates, this fraction (3.0 mg) was diluted with 4.2 mg of pure cholesterol acetate and repeatedly subjected to preparative TLC, adding more carrier as necessary and each time recovering the middle fraction from the previous chromatogram. After six such treatments, cholesterol acetate (2.7 mg, 1240 count/min) which was pure by thin-layer and gas-liquid chromatography was obtained. This was diluted with 33.9 mg of cholesterol acetate and hydrolyzed to cholesterol, which had a radioactivity of 9220 count/min per millimole. Two purifications through the dibromide lowered the radioactivity to 5430 count/min per millimole. Another such purification and recrystallization from ethanol-water did not change this activity.

Both of the plants studied contain sapogenins having 27 carbon atoms. Since cholesterol, unlike the other common plant sterols, also is a 27-carbon compound, the possibility exists of a biogenetic relationship with the sapogenins. In this connection it is interesting to note that we found neither cholesterol nor sapogenins in a sterol extract from *Xanthium pennsylvanicum*, although β-sitosterol and stigmasterol were present (*7*).

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Brown Fat: Thermogenic Effector of Arousal in Hibernators

Abstract. *Earlier indications that brown fat has a thermogenic role in rats exposed to cold suggested that certain comparable functions in hibernating marmots be investigated. From the results it appears that arousal of the animal by cold is induced by sympathetically activated thermogenesis in areas of brown fat so located, relative to the vasculature, that heat is transferred to the thoracic structures and cervical spinal regions.*

In previous reports (*1*, *2*) and discussions (*3*) evidence has been presented which indicates that multilocular brown adipose tissue in rats and other mammals has a thermogenic role. In experiments in the rat it has been shown that this thermogenesis is under autonomic control and becomes activated in response to an acute drop in ambient temperature. The brown fat, because of its location relative to the vascular system, is then able directly to heat the blood as it flows toward the thoracic organs and the cervical spinal regions. As applied to the hibernator (*2*), these findings give meaning to some early observations of Raphael Dubois (*4*), made in 1895, to the effect that the hibernating marmot could be rendered incapable of arousal, either through ligation of the subclavian veins or through spinal transection at segment C_4. Likewise, demonstration that brown fat has a thermogenic role in the hibernator would give metabolic support to the suggestion of Polimanti (*5*) that this tissue serves as a thermal insulator of the body cavity. Such a demonstration, moreover, would reinstate "hibernating gland" (*6*) as an organ discretely useful to the hibernator, a status largely moot since 1551 when Gesner first described the interscapular brown fat in the marmot (*7*).

Hence, the experiment reported here had a dual objective: to demonstrate (i) that thermogenesis could be elicited in vivo at sites of brown adipose tissue in a hibernator, and (ii) that the degree of this activity could be selectively enhanced by exposure of the hibernator to a critically low environmental temperature.

Our study was conducted at an altitude of 3800 m at the Barcroft Laboratory of the University of California's White Mountain High Altitude Research Station. Into each of three adult marmots *(Marmota flaviventris)* (Nos. 2, 5, and 6), deeply hibernating at ambient temperature of 6°C, iron-constantan thermocouples sealed into stainless steel hypodermic cannulae were inserted percutaneously into four sites of brown adipose tissue—the interscapular "hibernating gland," the mid-dorsal area between the paired superior cervical pads, and the axillary areas adjoining the left thoracic region and the right foreleg. Cannulae were inserted subcutaneously at "neutral" sites—a mid-dorsal site in about the T_{10}-L_2 region and also, in two of the marmots (Nos. 2 and 6), a mid-ventral site in the area of the xiphoid process. The cannulae were attached to a standard six-channel (Speedomax) recording device; deep rectal (about 7 cm) temperature and air temperature were read from calibrated thermistors. Respiratory rates were determined visually.

Observations were made first in the laboratory for 40 to 90 minutes at an ambient temperature of 6.8° to 7.0°C. Subsequently two of the animals (Nos. 2 and 6), were taken out-of-doors and