cholesterol *via* a thoracic lymph fistula produced an 8-10 fold increase in liver cholesterol synthesis from l-C^{14}-acetate.

Summary. An increased fecal excretion of cholesterol and related sterols followed the administration of H^3-β-sitosterol to rats receiving cholesterol-free emulsions containing bile salt and fatty acid. Approximately 53% of the administered labeled β-sitosterol was not recovered in the feces. Our study provides additional evidence that plant sterols are absorbed in the same manner as cholesterol and thereby compete with cholesterol for the sterol absorptive mechanism.

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Effects of Type of Restraint upon Heat Tolerance in Monkeys.* (23736)

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In the course of other experiments, monkeys were exposed to a warm environment while restrained on an animal board of the type ordinarily used for dogs and cats. Under these conditions, the rectal temperatures increased to an undesirable degree. The monkeys were able to reach equilibrium at an acceptable rectal temperature, however, with a different method of restraint; here the arms were held at the side of the body and the head was supported by a yoke around the neck. The observations are reported to call attention to the sensitivity of the temperature regulating mechanism of these animals to slight changes in the method of restraint.

Material and methods. Thirteen Macaca monkeys of both sexes ranging in weight from 3 to 6 kg, were used in 18 tests. Of the 6 exposed at 29°C ± 2°, the temperature of the animal quarters, 3 monkeys were tested with each type of restraint; heart rates were recorded on 2 of the 3. Two of the other monkeys were also exposed at 38°C, one with each type of restraint. The remaining 7 monkeys were exposed once at 38°C. This temperature was controlled to ± 0.5°C and the relative humidity was 45% ± 2%. The critical features of the 2 types of restraint, both with animal in prone position, are as follows: *Arms forward*: The animal rested with head free on a solid wooden platform, tied by wrists and ankles, with arms extended beyond the head. The knees and elbows had little freedom to bend. *Arms backward*: The animal rested on a platform of 0.5 in. wire mesh with an air space of 1.5 in. between it and the table. The body was held in position by yoke around the neck and by tying the arms at wrist and elbow along the side of the body. The legs were tied at the ankles but were only held loosely. At 29°C rectal temperatures were recorded with a Yellow Springs Tele-Thermometer thermistor and heart rate was obtained with a Burdick Electrocardiograph. At 38°C a cop-

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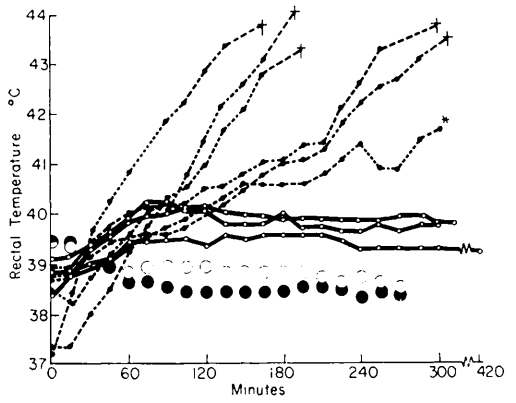

FIG. 1. Course of rectal temperature during restraint. Individual monkeys at environmental temperature of 38°C are shown by dashed lines in "arms forward" position and by solid lines in "arms back" position. Group averages at environmental temp. of 29°C are shown by closed circles in "arms forward" position and by open circles in "arms back" position. Mean rectal temp. and range of variation during entire period of restraint for individuals at 29°C were: in "arms forward" position, 37.4 ± .5, 37.8 ± .7, 39.2 ± 1, 38.5 ± .4 and 38.7 ± .5; in "arms back" position, 37.6 ± .9, 39.2 ± .4, 39.6 ± .2 and 38.8 ± .7°C.

per constantan thermocouple and a Leeds and Northrop Speedomax recorder were used to obtain the rectal temperature. The thermocouple or thermistor was inserted to a depth of 6 to 8 cm and recorded to the nearest 0.05°C.

Results. In nearly 5 hours at 29°C the type of restraint had a negligible effect on rectal temperature and heart rate. In Fig. 1, the mean temperature declined in the first hour and then remained nearly constant through the fourth hour. The mean heart rate for the entire experiment was 255 beats per minute for the "arms back" restraint and 254 beats per minute for the "arms forward" restraint. The range of the heart rate during this time was from 210 to 290 beats per minute.

At 38° the rectal temperatures of individual monkeys are shown in Fig. 1. With "arms back" restraint the temperature increased about 1°C in the first hour and then remained nearly constant through the sixth hour. These monkeys maintained a fresh and active attitude throughout this period. With "arms forward" restraint, the temperature rose progressively and the monkeys became unresponsive after about 2 hours.‡

Discussion. The ability of the restrained monkey to maintain thermal equilibrium under heat stress, appears to depend on differences in the method of restraint that have not been identified. Two possibilities may be mentioned; one, that the raised wire-mesh screen in the "arms back" restraint favored heat loss by evaporation; the other that the "arms forward" method was less acceptable to the animals with the result that they struggled more, increasing the heat output, or through an emotional response, restricted the peripheral circulation, decreasing the sensible heat loss. That these monkeys do not take kindly to restraint is indicated by the elevation in heart rate at 29°C above what has been reported(1) under "resting conditions" (average 192, range 165-240), and by the hyperglycemia reported by Poirier *et al.*(2). The lability of the vasomotor system to strong emotion has been recognized at least since the work of Hill(3). Whatever the cause, it is evident that the ability of these animals to withstand heat stress is critically affected by the method of restraint.

Summary. Macaca monkeys attached to an animal board by means of the wrists and ankles with arms and legs extended, in an environment of 38°C and 45% relative humidity, were unable to maintain their temperature equilibrium. The rectal temperature increased steadily to over 43°C in a period of from 3 to 5 hours. When restrained on a wire mesh platform by means of a neck yoke and attachment of the arms along the side of the body, the monkeys held a steady rectal temperature of about 40°C for 5 hours. At a room temperature of 29°C equilibrium of rectal temperature was maintained with both types of restraint with average heart rates over a period of 4.5 hours of 254 for one type and 255 for the other. It is concluded that the manner in which a monkey is restrained can modify its physiological responses during exposure to heat stress.

‡ The monkey whose record in Fig. 1 is marked with an asterisk was petted and given water to drink during exposure.

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Viability of Monkey Kidney Tissue Cultures Stored at 5°C. (23737)

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The widespread use of monkey kidney cells for production and study of mammalian viruses has created a demand for this animal which is becoming difficult to meet. Prolongation of the useful life of tissue cultures would be one measure to alleviate this situation. Monkey kidney epithelial cells generally can be maintained in a usable condition for only 3-4 weeks at 37°C. This period has been extended somewhat by storing cultures at room temperature. An early report by Fischer(1) described such a procedure for the storage of chick tissue cultures. In addition to extending the life of monkey kidney tissue cultures, there is an obvious advantage in studying a small number of cultures for non-specific degeneration, foamy, and simian agents, before the remainder of the cultures are used for vaccine safety testing or for virus research purposes.

It has been observed that storage of monkey tissue cultures at 4-6°C provides a satisfactory procedure for relatively short-term preservation of large numbers of cultures. The following report describes such observations along with studies of the sensitivity of stored cultures to poliovirus.

Methods. Kidneys were removed from exsanguinated 5-7 pound rhesus monkeys and trypsinized as generally outlined by Youngner (2). The cortical tissue from 4 sets of kidneys was minced, pooled, and washed 3-4 times in 3 volumes of Earle's salt solution containing penicillin and streptomycin (100 units and 100 μg, respectively). The washed tissue was trypsinized with prewarmed (37°C) 0.25% trypsin in Earle's salt solution in a blendor jar for 10-minute intervals at a speed just below the foaming level of the trypsin. Usually 4 extractions were made with 20-25 ml of trypsin solution per kidney decanted and replaced at each interval. The trypsinized cells were pooled and centrifuged for 15 minutes at 10 g, following which they were washed 3 times in Earle's salt solution containing 2% calf serum and centrifuged for 10 minutes after each wash. The cells were transferred to a calibrated conical centrifuge tube and centrifuged at 10 g for 3-4 minutes after which they were ready for resuspending in nutrient media. Medium 199(3) was prepared by diluting a 10 X concentrate with sterile demineralized water. The pH was adjusted to approximately 7.4 with a 5% solution of sodium bicarbonate (usually about 25 ml of bicarbonate solution per liter of diluted Medium 199). The adenosine triphosphate and labile compounds were added just before use of the medium. Penicillin and streptomycin were added to a final concentration of 100 units and 100 μg, respectively. All Medium 199 used for monkey kidney cultures contained 2% calf serum (heated 56°C for 30 min.) for initial growth of cells and 1 or 2% calf serum for maintenance of cells. Packed cells were resuspended in Medium 199 containing 2% calf serum at a ratio of 1 ml of cells to 200 ml of media. This suspension usually contained about 400,000 cells per ml. Tubes were inoculated with 1.0 ml of suspension, 2-ounce bottles with 6 ml, 8-ounce bottles with 20 ml, and 32-ounce

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