been evidence of a fever. However, in contrast to the fever syndrome in which respiratory frequency (RF) is greatly depressed, the RF of this sheep at 20°C ambient temperature (T_a) was within the normal range (60–80 breaths/min). A local increase of T_{hy} by 1.5°C evoked panting (200 breaths/min) and a fall in T_{re} to about 40.5°C. Cessation of hypothalamic heating was followed by a fall in respiratory frequency to its pre-heating level, but T_{re} remained at its new level. A second period of hypothalamic heating again caused panting and a fall in T_{re} to 39.5°C at which level it remained even after hypothalamic heating was stopped. This observation indicated a disturbance to the set-point mechanism but no disturbance to the pathway from warm-sensors to heat-loss effectors.

A neuronal model of the pathways between temperature sensors and thermoregulatory effectors has been based on the thermoregulatory effects of intraventricular injections of putative transmitter and related substances[9, 8, 10]. The effects in this sheep of 2 of these substances, 5-hydroxytryptamine (5-HT) and eserine, were unaltered by the lesion but there was no recovery from the displacements of T_{re} which they produced. An intraventricular injection of 300 µg 5-HT caused the expected peripheral vasodilatation, increase in RF and a fall in T_{re} which persisted after the other effects of 5-HT had passed. An intraventricular injection of 300 µg eserine caused the expected peripheral vasodilatation, onset of shivering, decrease in RF and a rise in T_{re} which again persisted after the other effects of the injection had passed. These observations were considered to be evidence of the integrity of hypothalamic synapses in the neural pathways from temperature sensors to thermoregulatory effectors, and of the pathways from these synapses to the effectors.

Since pyrogens are considered to interfere with set-point mechanism and the set-point mechanism appeared to be impaired in this animal, we gave an i.v. dose of TAB vaccine sufficient to evoke a fever response in a normal sheep. No fever developed. In fact, the response was the converse to fever: RF increased and T_{re} fell slightly.

For this syndrome to be attributable simply to the inactivation of the temperature set-point mechanism, the initial observation of a high T_{re} must be explained. The critical T_a of the fully fleeced sheep is below 5°C, so at the temperature of the animal house (15–20°C) a normal sheep is probably actively losing heat to remain in thermal balance. In the absence of an effective set-point mechanism and little or not active heat loss, core temperature might be expected to rise until passive heat loss equals heat production. To test this explanation, the sheep was shorn. In this condition, T_a of 15–20°C is below thermoneutrality, and some shivering was present, but not sufficient to prevent T_{re} from falling below the normal range. When T_{hy} was raised the shorn animal ceased to shiver and there was a further fall in T_{re} to below 38°C. There was no active recovery of T_{re} after hypothalamic heating had ceased.

On the 68th day after the surgery, the sheep died quite suddenly although, apart from the impairment of temperature control, the animal had seemed in good health. A post mortem examination revealed extensive lesions in the anterior hypothalamus immediately caudal to the rear pair of thermodes.

Our conclusion is that by a chance circumstance which may not be readily repeatable, we had produced a hypothalamic lesion which inactivated or seriously disturbed the temperature set-point mechanism without causing a detectable interference with the pathways from temperature sensors to thermoregulatory effectors. This indicates that the normal set-point mechanism, and the pyrogen induced interference with normal body temperature, depend on structures other than the primary hypothalamic temperature sensors and the pathways from these sensors to thermoregulatory effectors. However, this syndrome is not consistent with the concept of a set-point signal which exerts an inhibitory influence on the sensor-effector pathways, since the removal of such an influence would be expected to increase rather than decrease the responsiveness of the thermoregulatory processes to changes in body temperature.

Zusammenfassung. Aus diesem Versuch wird geschlossen, unbeabsichtigte hypothalamische Läsion durch Thermoden-Implantation verursacht zu haben, die beim Schaf auffallende thermoregulatorische Störungen hervorriefen, so dass der thermoregulatorische Sollwert-Mechanismus ausgeschaltet wurde, die Bahnen zwischen den hypothalamischen Thermosensoren und den thermoregulatorsichen Effektoren hingegen durch die Läsion nicht beeinträchtigt waren, was eine anatomische Differenzierung zwischen Sollwert-Mechanismus und Sensor-Effektor-Bahnen erlaubt.

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Isolation of Large Sheets of Frog Skin Epidermis

Studies of active transport using intact isolated frog skin[1] have been hampered by the presence of the corium which constitutes not only a diffusion barrier[2], but also a metabolically active store of electrolytes. Previous efforts[1, 3–6] to split off the epidermis of frog skin has resulted in only small pieces. We have isolated large sheets of epidermis by trypsin hydrolysis. The problem was the tela subcutanea which is virtually impermeable to bovine serum albumin (Lindley and Hoshiko, unpublished).

Methods. The tela subcutanea of abdominal skin of *R. pipiens* was lightly scored with a scalpel to make a grid of fine cuts. The skin was mounted in an open cylindrical chamber (with the corium facing into the chamber) much like a piece of cloth in an embroidery hoop. The chamber was placed with the epithelial surface down in a petri dish containing sulfate solution (55 mEq/l Na_2SO_4, 5 mEq/l K_2SO_4, 5 m*M tris* buffer, pH 7.8) bubbled with compressed air. The corium was covered with sulfate solution contain-

ing trypsin (80 U/ml, Worthington) and incubated at room temperature (22°C). After 30 to 60 min the corium was washed with fresh sulfate solution to remove trypsin. The chamber was inverted to expose the epithelium which was cut around the periphery with scissors and floated off.

Results and discussion. Pieces up to 5 cm in diameter have been made. The isolated epidermis was sandwiched between nylon net screens for mounting in flux chambers. The potential fell slightly upon scoring but recovered during trypsin incubation. Mean potential before removal of the epithelium was 102 mV and 84 mV 15 min after (Figure 1). Short circuit currents were comparable to those of isolated intact skins. If trypsin incubation is longer than 30 min in summer or 60 min in winter, sulfate leakage occurs although potentials and currents are maintained (Table). Further incubation decreased the potentials and currents. Whereas intact skins maintained their potentials in sulfate solution, the split skin required calcium (0.5 mEq/l) in the inside bathing medium.

Sulfate permeability of isolated epithelium

Date	Digestion time (h)	Potential (mV)	Sulfate permeability
October 21	1	66	0.69×10^{-8} cm sec^{-1}
June 23	0.5	32	0.04×10^{-8}
June 23	1	62	0.156×10^{-4}

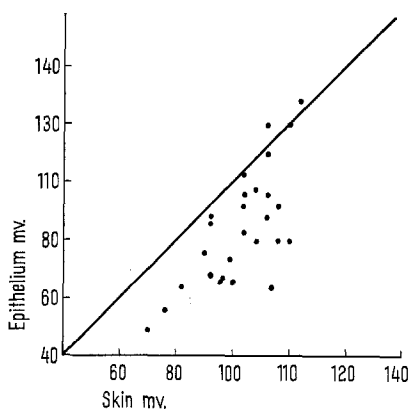

Fig. 1. Open circuit potentials of excized frog skin before removal of the epidermis (abscissa) is plotted against the potential of that isolated epidermis 15 minutes after removal (ordinate).

Figure 2 shows the transition from the intact to the 'split' skin. Cleavage occurs at the basement membrane with the stratum germinativum going with the epithelium. The stratum germinativum stains (toluidine blue) less well than either the other layers beside it or the stratum germinativum in the intact portion of the skin. After longer trypsin treatment, the stratum germinativum was absent. Lightly staining 'mitochondria-rich cells'[7] are also seen in the middle layers. In the split epidermis, no mucous glands are evident and one gland has remained in the isolated corium. On the dermal surface of the epidermis, numerous remnants of glands are seen, with both light microscopy and scanning electron microscopy. The density is about 65 glands/mm^2, not far from the density of 70 /mm^2 reported by ENGLEMANN[8] for intact skin. Apparently the body of the gland is gone and only a stump of the duct remains. An occasional mucous gland (cf. Figure 1, Ref.[4]) but no serous glands have been seen.

Sulfate permeability of the split skin compares favorably with those reported by USSING[9] for intact skin of 2.5×10^{-8} cm sec^{-1}. Although skin gland ducts have been disrupted in the split skin, apparently the sphincters are tightly closed. MALAMED and LINDLEY (personal communication) have found that the sphincter consists of a single doughnut shaped cell closed upon itself with tight junctions.

We have used the split skin preparation to study the kinetics of Na-22 washout[10].

Zusammenfassung. Nach Trypsinbehandlung abgetrennte Froschepidermis von bis zu 5 cm ⌀ zeigt dieselbe Potenialdifferenz, Stromkurzschluss- und Sulfatpermeabilität wie die unveränderte Froschhaut. Die präparierte Epidermis besitzt fast keine Hautdrüsen, jedoch fast völlig verschlossene Drüsenausführungsgänge.

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Fig. 2. Cross-section of skin treated with trypsin and epidermis removed in one part. Calibration line is 100 µm.

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