bility that the adrenals are near to a state of maximum stimulation.

References

1. CARPENTER, R. K., MACLEOD, L. D., and REISS, M. J. *Physiol.*, **105**, 231 (1946).
2. TEPPERMAN, J. *Endocrinology*, **47**, 384 (1950).
3. MCKEE, R. W., and WALKER, J. K. *Federation Proc.*, **10**, 219 (1951).
4. HECTER, O., *et al. Recent Progr. Hormone Research*, **6**, 215 (1951).
5. HAYNES, R., SAVARD, D., and DORFMAN, R. I. *Science*, **116**, 690 (1952).
6. MCKEE, R. W., and LONBERG-HOLM, K. (In press.)
7. ANFINSEN, C. B., *et al. J. Exptl. Med.*, **84**, 607 (1946).
8. KREBS, H. A. *Biochem. J.*, **31**, 1633 (1935).
9. MCKEE, R. W., COBBEY, T. S., JR., and GEIMAN, Q. M. *Endocrinology*, **45**, 21 (1949).
10. LONG, C. N. H. *Federation Proc.*, **6**, 461 (1947).
11. NADEL, E. M., and SCHNEIDER, J. J. *Ibid.*, **11**, 263 (1952).

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A New Technique for the Study of the Effects of X-Radiation on Mammalian Skin Maintained at Different Temperatures During Exposure[1, 2]

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Evidence relevant to the problem of the radiosensitivity of protoplasmic systems in relation to the temperature prevailing therein during exposure to ionizing radiations has been recently and fittingly characterized as "equivocal" (*1*). In addition to its theoretical importance, the issue, as it relates specifically to mammalian skin, is not without practical implications (*2*). Mammalian skin, subjected to reduced temperatures during exposure to x-radiation, exhibits increased radioresistance (*2, 3*); conflicting observations have been reported (*4*). The intricacies attending the general problem have been well outlined by Henshaw and Francis (*5*).

Heretofore, investigations on mammalian skin in this connection have employed techniques to which have been attached important disadvantages (*3, 6*); they may be summarized as follows: (*a*) for want of adequate shielding the radiation passes through the animal and affects considerably more than the skin, with the accompanying probability of significant indirect effects of the radiation on the skin; (*b*) the change in temperature induced by cold applications and ligation, while directed primarily to the skin, involves, in fact, a reduction in over-all metabolic level of large parts of the animal not directly under study; (*c*) such methods are chiefly effective only when applied to newborn mammals, which lack adequately developed temperature-regulating mechanisms (*2*); (*d*) the study of radiosensitivity of cold and warm areas of skin has, by and large, involved a study of responses in respectively different individual animals (i.e., response of cold skin in one animal and of warm skin in another) and, no less importantly, a spreading of controls among different animals; such a procedure does not reckon well with the real and vexing problem of individual variations in response to given conditions of irradiation.

It seemed of value to develop a technique calculated to circumvent the disadvantages and limitations outlined above and thereby pave the way for better controlled and less limited experiments. The basic features of such a technique are herein described. The method has been effectively proved in our laboratory; it is practicable for almost any type of homeotherm or larger poikilotherm. We believe that it will be interesting to those engaged in similar studies.

The technique is as follows (mice and rats employed principally): The animal is anesthetized with sodium Nembutal. Subsequently, two parallel, anteroposterior, skin-deep incisions are made, equidistant from the midline, on the back of the animal (about 6 cm in length and 5 cm apart in the rat). The skin flap between the incisions is separated from the underlying skeletal muscles. Beneath this loose flap of integument a hollow lead chamber is inserted (Figs. 1 and 3); by means of this element the cooling or warming of a definite area of skin is accomplished. The chamber is elevated so that its lower surface does not contact the underlying tissues; this involves only

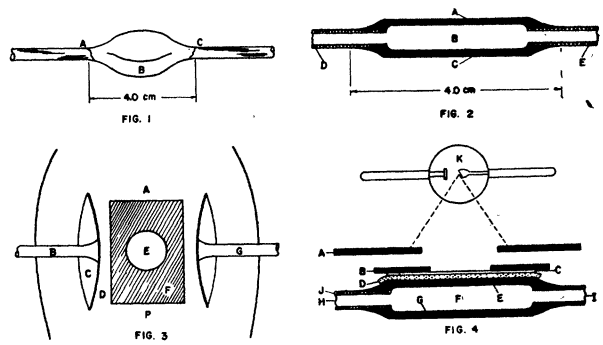

FIG. 1. Dorsolateral view of temperature-regulating chamber. *B*, chamber proper; *A, C*, junction of brass tubing inlet and outlet.

FIG. 2. View in longitudinal section of Fig. 1. *A, C*, upper and lower walls of lead chamber; *B*, interior of chamber; *D, E*, points of attachment of rubber tubing.

FIG. 3. Dorsal view of chamber in place. *A, P*, anterior and posterior on the animal; *B, G*, inlet and outlet; *C*, incision; *D*, region of lower left quadrant of separated flap of back skin; *F*, lead plate with aperture allowing exposure of skin area, *E*.

FIG. 4. View, in longitudinal section, of final arrangement. *K*, x-ray tube; *A*, lead plating protecting the animal proper; *B*, corresponds to *F* in Fig. 3; *C*, thin strip of plasitc used, optionally, to enhance contact of skin with tepmerature-regulating element; *D*, skin; *E, F, G*, upper wall, interior, and lower wall, respectively, of chamber; *J, H, I*, wall of brass tubing, inlet and outlet.

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a slight stretching of the skin. The upper and lower walls of the chamber provide a thickness of lead sufficient to preclude penetration of x-rays below the chamber; thus the radiation is confined exclusively to the skin (Figs. 2 and 4). To the chamber are attached brass tubes, serving as inlet and outlet, for the circulation of water; the temperature of the water determines the temperature of the chamber. The circulation is effected by a simple siphoning arrangement.

The chamber, with brass tubing attachments, is supported by clamps, and the animal is slipped into position; rubber tubing is then attached to the inlet and outlet, and the circulation of water is begun. A piece of lead plating with a small circular aperture is then placed immediately upon the skin area overlying the chamber (Fig. 3). The skin is thus brought into fixed apposition with the surface of the temperature-regulating device; the aperture leaves a definitely circumscribed area of the skin to be exposed to the radiation. The skin is conditioned to the temperature prevailing in the system for a period of 10–15 min before irradiation. We have ascertained that the temperature of the skin very closely approximates the temperature of the lead surface upon which it rests. The cooling of skin is enhanced by the ligating effect of the lead plate upon the cutaneous blood vessels. The entire animal, with the exception of the small circular area of skin to be irradiated (Fig. 3, *E*), is shielded by a large lead plate.

In our early experiments the irradiation of warm and cold areas of skin was effected in respectively different animals; similarly the controls. However, we have adapted the technique of a tandem arrangement of two pairs of chambers to make it possible to produce, simultaneously, on one and the same experimental animal, in symmetrical pattern, two cold skin areas (approx. 5° C) and two warm skin areas (approx. 40° C); one of each pair serves as an experimental area and the other as the control. At temperatures we have employed, we have never encountered any untoward responses of control areas. After irradiation the incisions are closed with nickel silver wound clips.

The advantages of this technique may be summarized as follows: (*a*) the radiation is strictly confined to a definitely localized region of the skin; (*b*) the regulation of temperature is similarly localized; (*c*) the warming and cooling of skin areas can be simultaneously effected in one and the same experimental animal, with, in addition, the appropriate controls on the same animal; (*d*) the use of the basic features of the method are desirable even when there is no concern with the experimental regulation of the temperature of the skin; (*e*) one need not, with this technique, be confined to the use of newborn mammals; (*f*) the technique can profitably be employed in the study of elements other than mammalian integument and, it would seem, could be adapted to subserve profitably the analysis of a variety of radiobiological problems.

Details regarding the use of this technique, and evidence deriving from our studies on the radiosensitivity of mammalian skin in relation to temperature, will be published elsewhere.

References

1. NICKSON, J. J. *Symposium on Radiobiology.* Comment, H. M. Patt, p. 213 (1952).
2. EVANS, T. C., and KERR, H. D. *Am. J. Roentgenol.*, **50**, 629 (1943).
3. EVANS, T. C. *Ibid.*, **45**, 888 (1941).
4. MOTTRAM, J. C. *Brit. J. Radiol.*, **8**, 32 (1935).
5. HENSHAW, P. S., and FRANCIS, D. S. *J. Cell. Comp. Physiol.*, **7**, 173 (1935).
6. CHRISTENSEN, W. R., SOMMERS, S. C., and SPAULDING, C. K. *Am. J. Roentgenol.*, **68**, 801 (1952).

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Centrifugal Preparation of Rat Liver Mitochondria Free of Microsomes[1]

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The isolation in bulk quantities of cytoplasmic particulates from rat liver cells by the differential centrifugation technique, originally described by Claude and further developed by Hogeboom *et al.* (*1*), is being widely applied in the study of cell physiology. In the preparation of the mitochondrial fraction, several investigators have called attention to the occurrence of a loosely packed material which sediments over the more compact mitochondrial pellet at one stage of the fractionation. Thus Muntwyler *et al.* (*2*) emphasized the need for removal of this material from the mitochondria. They reached the conclusion that it was more properly a part of the microsomal fraction, largely on its microscopic appearance and on the relative nitrogen and pentose nucleic acid content of the mitochondrial and microsomal fractions with various partitionings of the intermediate material. Schneider and Hogeboom (*3*) reaffirmed their original mention of the errors introduced by not removing this material from the mitochondria, i.e., too much nitrogen and pentose nucleic acid in the latter and too little in the microsomes. Most recently, Potter *et al.* (*4*) stated the likelihood that the loose material is probably microsomal rather than mitochrondrial in nature, based on its staining properties.

The present study was made to devise a procedure for a more complete separation of the intermediate fraction from the mitochondria. Also, additional evidence has been obtained that this fraction is part of the presently accepted microsomal fraction, and should be incorporated with it.

When centrifugation of the mitochondrial fraction is carried out at 15,000 × gravity by the usual method (*1*) in a large, e.g., 2.5-cm diameter tube it is difficult to see the boundary between the loose upper layer and

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