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.

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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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the mitochondrial layer, and a sharp separation is not possible. In order to overcome this difficulty, only the clear supernatant liquid, containing a large proportion of the microsomes, is removed and the entire sediment is transferred to a smaller, 9-mm diameter lucite tube. The mixed material is resuspended in about 3 volumes of either 0.25 *M* or 0.88 *M* sucrose solution with the aid of a pestle made from a small piece of glass tubing with a bubble blown on the end to fit the lucite tube. Upon recentrifugation at 15,000 × gravity for 15 min (30 min if 0.88 *M* sucrose is being used), a pinkish white material is loosely layered over the sharp mitochondrial boundary. This material is easily removed by means of a fine capillary pipet, care being taken to remove no mitochondria. The mitochondria are resuspended and the process is repeated until no more loose material appears over the mitochondria. The washings, including the loose material, are added to the clear supernatant liquid removed in the first step described above, and the whole is centrifuged at high acceleration, e.g., 100,000 × gravity, to separate the microsomal fraction, if desired.

It was found that at least 3 or 4 washes are usually required to remove the intermediate material completely from the mitochondrial fraction. In the first unwashed mitochondrial sediment this material occupies about 60–70% of the whole pellet volume, and after 1 wash it still represents 40–50%. These proportions were determined by centrifugation in capillary tubes approximately 3 mm in diameter to give an elongated pellet.

The intermediate material could be conveniently collected separately by this method, and its properties were compared with those of purified mitochondrial and microsomal fractions in the following experiments.

(a) A suspension of the intermediate material was centrifuged at high acceleration into a compact pellet. The pellet had the characteristic appearance of a microsome pellet, a pink gel, with a trace of buff mitochondria at the bottom.

(b) Electronmicrograms of the intermediate material, while revealing aggregation of the constituent particles, showed that the particles were identical in size and shape with the microsomes.

(c) An optical ultracentrifuge sedimentation pattern of a suspension of the intermediate material centrifuged at 260,000 × gravity was compared with one of a microsome suspension. The patterns appeared identical, whereas purified mitochondrial suspensions give no pattern at all with these optics.

(d) Staining of the mitochondria, intermediate material, and microsomes separately by the method of Potter *et al.* (*4*) confirmed their findings. The mitochondria exhibited staining properties clearly different from those of the intermediate material and microsomes, while the latter were the same.

(e) The total nitrogen content of duplicate aliquots of the purified mitochondria from a rat liver was found to be higher than that of the intermediate material or the microsomes from the same liver, which were very similar, as may be seen in Table 1.

TABLE 1

TOTAL NITROGEN CONTENT OF VARIOUS LIVER CELL FRACTIONS

Fraction	Nitrogen content (g N/g dry sample*)	
	Sample 1	Sample 2
Mitochondria	0.124	0.124
Intermediate material	0.104	0.102
Microsomes	0.097	0.095

* Each fraction was washed once with distilled water to remove the bulk of the sucrose.

(f) Two rats were injected with a tracer dose of radiophosphorus-labeled phosphate and sacrificed after 7 hr. The nitrogen and phosphorus content, total phosphorus specific activity, and phospholipid phosphorus specific activity of the purified mitochondria, intermediate material, and microsomes were determined. As may be seen in Table 2, the mitochondria stand in sharp contrast to the intermediate material and the microsomes in every respect, whereas the latter two fractions are almost identical in their properties.

TABLE 2

N/P RATIO AND P SPECIFIC ACTIVITIES OF VARIOUS LIVER CELL FRACTIONS

Fraction	g N/g P of fraction		Specific activity of total P		Specific activity of phospholipid P	
	Rat A	Rat B	Rat A	Rat B	Rat A	Rat B
Mitochondria	13.5	12.9	0.41	0.51	0.38	0.50
Intermediate material	5.3	5.8	0.33	0.39	0.46	0.54
Microsomes	5.9	5.3	0.34	0.39	0.47	0.55

On the basis of these experiments, which included the examination of physical, chemical, and metabolic properties of the intermediate material, it is concluded that the loose upper layer sedimenting with the mitochondria is definitely microsomal in character and should be removed completely from mitochondrial preparations from rat liver cells. A procedure is outlined for effecting this end, and consists largely of several recentrifugations of the mitochondrial fraction.

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