and *Lactobacillus arabinosus* (*5*), but more recent work has cast doubt upon the occurrence of this reaction in the latter organism (*6*). It was postulated that tryptophan was formed by the condensation of indole and serine in spinach leaves, but no experimental evidence has been presented (*7*).

Studies in this laboratory, devoted to the biogenetic relationship between tryptophan and lysergic acid, revealed that the mycelium of *Claviceps purpurea* (Fries) Tulasne possessed considerable tryptophan desmolase activity. The strain of *Claviceps* used was isolated from a sclerotium of barley ergot and subjected to a process of physiological adaptation until it was capable of prolific mycelial development in submerged culture. The medium used consisted of a basic mineral nutrient solution (*8*) containing 2% mannitol and 0.1% each of *dl*-alanine, *l*(−)-asparagine, *l*(+)-aspartic acid, *l*(+)-glutamic acid, *l*(−)-leucine, and *dl*-valine.

Mature mycelium (7–10 days growth) was collected, washed with distilled water, and portions (ca. 50 mg dry weight) were transferred to test tubes containing approximately 100 γ of indole and 2 mg of *dl*-serine in 2 ml of water. *M*/15 phosphate buffer was then added to produce a total volume of 5 ml in each tube. The tubes were agitated on a reciprocal shaking machine at room temperature for periods up to 8 hr. Ten milliliters of toluene was pipetted into each tube and shaken vigorously in order to stop the reaction by removal of unutilized indole. The tubes were then centrifuged, filtered, aliquots of the toluene layer assayed for indole by the process of Wood *et al.* (*9*), and aliquots of the aqueous layer for tryptophan by the method of Nason *et al.* (*10*). Control tubes containing washed *Claviceps* mycelium and water failed to show any accumulation of indole or tryptophan. The results of a series of analyses are reported in Table 1. Tryptophan formation correlated with indole disappearance, and yields of nearly 90% of theory were obtained. The product was further characterized by spotting quantities of the aqueous layer of tubes showing considerable tryptophan accumulation on strips of Whatman No. 1 filter paper and chromatographing with butanol-acetic acid-water (*11*) and methanol-butanol-benzene-water (*12*). From the qualitative data obtained it was concluded that *Claviceps* has ability to utilize indole and serine to produce tryptophan under the conditions described.

The study of *Claviceps* metabolism is being continued, with special emphasis upon other possible tryptophan precursors, as well as upon products formed by tryptophan utilization.

TABLE 1

INDOLE UTILIZATION AND TRYPTOPHAN FORMATION
(Indole added = 96 γ/tube)

Reaction time (hr)	1	2	4	8
		Indole utilized (γ)		
Tube 1	19	39	91	96
2	14	35	91	96
3	15	38	90	96
Av	16	37	91	96
		Tryptophan formed (γ)		
Tube 1	24	51	81	154
2	18	51	90	146
3	20	48	95	138
Av	21	50	89	146

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Oxygen Consumption of Adrenal Slices from Normal and Scorbutic Guinea Pigs and the Influence of Added ACTH[1, 2]

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Oxygen consumption by slices of adrenal cortex has been used as a criterion of cortical cellular metabolism by several groups of workers (*1–3*). Carpenter *et al.* (*1*) observed an increase in oxygen utilization and of aerobic lactic acid production by the adrenal cortices of rats treated with large doses of ACTH. Tepperman (*2*) found when purified ACTH was added *in vitro* to slices of dog adrenal cortex that the oxygen consumption of the tissue was increased and its ascorbic acid content depressed. Hecter *et al.* (*4*), working with whole perfused cow and hog adrenal glands and Haynes and co-workers (*5*), utilizing slices of adrenals have demonstrated an accelerated synthesis of 17-hydroxycorticosterone and of formaldehydogenic substances by the *in vitro* addition of purified ACTH.

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[2] The authors wish to thank Ellen L. Marston for technical assistance in this study.

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In this study an isotonic inorganic medium and respiratory system was developed whereby maximum and constant oxygen consumption values were obtained. This system was then utilized for determining (a) the respiratory activity of various portions of the guinea pig adrenal gland, (b) the total oxygen utilization of the glands from normal and scorbutic animals, and (c) the changes in oxygen consumption with *in vitro* addition of ACTH.

The isotonic suspending medium employed was the same as that used for investigations on Ehrlich's ascites carcinoma cells (*6*) and was modified from that employed in the studies on malaria parasites (*7*). The composition of the medium, in grams per liter, is: $NaH_2PO_4 \cdot H_2O$ 1.5185, Na_2HPO_4 6.2480, NaCl 3.9370, KCl 0.4100, $MgCl_2 \cdot 6H_2O$ 0.0950, $CaCl_2$ 0.0560, $MnCl_2$ 0.0020. Variations in the inorganic composition of the medium were investigated and it was determined that halving or doubling the concentration of Ca^{++}, Mg^{++}, and Mn^{++} did not alter the oxygen consumption of the adrenal slices. Likewise, small increments of the K^+ content of the medium had little or no effect; however, increasing the concentration, from 5.5 mM/l to 110 mM, and compensating for the change by decreasing the Na^+, diminished the oxygen utilization by 15–25%.

The most important technical aspects of this problem are the rapidity and care with which the adrenal slices are prepared. Chilling and keeping the glands cold is not necessary providing one works rapidly, and rigorously avoids drying and fragmenting the tissues. Thus upon sacrificing the animal the adrenals were immediately removed, dissected free of fat and connective tissue, and placed in a Petri dish containing a piece of filter paper moistened with the isotonic suspending medium.

Slicing of the gland was carried out rapidly, using a 0.5-mm Stadie-Riggs hand microtome slicer containing a disk of ashless filter paper moistened with the isotonic medium. The glands were cut into 4 slices, each about 0.5 mm thick, the outer 2 being principally cortical tissue while the inner 2 slices were mixtures of cortical and medullary tissue. As each slice was removed from the slicer it was placed on parafilm paper, quickly weighed on a Roller-Smith torsion balance, and immediately placed in a small size (7 ml) Warburg vessel containing 0.75 ml of suspending medium and 0.1 ml of 6% glucose solution, and with 0.1 ml of 15% potassium hydroxide and an accordion (15 × 20 mm) of Whatman 40 filter paper in the center well. The vessels were placed on the manometer in the 38° C bath, flushed with 100% oxygen and equilibrated for 10 min. Oxygen consumption was measured for a period of 3 hr and the values reported here are for the second hour which, in most cases, was the most linear portion of the curve. From 20 to 40 mg of tissue were employed for each vessel. The left adrenal was used for respiration studies and the right gland for dry weight determinations. The oxygen consumption values for the whole glands were obtained by summing the values for the 4 individual slices.

Glucose, glycerol, and sodium lactate were tested as substrates for respiration. Glucose was found to be superior and routinely used. Without any added substrate the oxygen utilized was about 20% less than when glucose was added.

Cyanide (*8*), inhibited oxygen consumption 15, 68, and 87% at concentrations of 10^{-4}, 10^{-3}, and 10^{-2} *M*, respectively. Ascorbic acid added to the system did not change the oxygen uptake.

Employing the above described system and observing the indicated precautions, it was possible to obtain maximum and reproducible values for oxygen consumption. The animals were made scorbutic as described in a previous publication (*9*) and were used after 21–28 days on the scorbutic diet. It is of considerable interest that the Q_{O_2} values for the scorbutic guinea pig adrenal glands are nearly twice those for normal glands (Table 1). Although not given in the table, values for the individual tissue slices showed the oxygen consumption for the slices of cortex to be more than twice that for the center gland slices composed of mixed medullary and cortical tissue (137 and 57 mm^3/100 g wet weight, respectively).

Table 1 indicates the *in vitro* effect of adding 2 mg of ACTH per Warburg vessel. There is with the normal adrenal tissue a 55% increase in oxygen consumption; whereas with the scorbutic tissue, there is no increment of oxygen utilization. The reason for the lack of augmentation of respiration in the scorbutic gland is not apparent; however, it is possible that the tissue is already stimulated maximally. Long (*10*) has obtained evidence of increased cortical hormone activity following the injection of ACTH into guinea pigs kept for 14 to 16 days on a scorbutic diet. However, the report by Nadel and Schneider (*11*) which showed an increased excretion of formaldehydogenic substances by the scorbutic guinea pig in an advanced state of vitamin deficiency is indicative of the possi-

TABLE 1

In vitro EFFECT OF ACTH* ON OXYGEN CONSUMPTION OF NORMAL AND SCORBUTIC GUINEA PIG ADRENAL SLICES

Whole adrenal slices	Oxygen uptake		Dry weight of glands
	(mm^3/hr/100 mg wet weight)	(Q_{O_2})	(%)
Normal	86 (7)†	2.77	31 (5)
Normal + ACTH	125 (2)	4.03	—
Scorbutic	141 (7)	5.63	25 (5)
Scorbutic + ACTH	149 (2)	5.96	—

* Two milligrams of Armour's ACTH, No. 212-74, 45% of activity of La-1-A, were added per vessel. We are indebted to Edwin E. Hays of the Armour Research laboratories for this material.

† The total numbers of animals used in these studies are given in parentheses. The values are average figures for the adrenals (animals), each being obtained from 4 slices. Variations among glands were no more than ± 10%.

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

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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

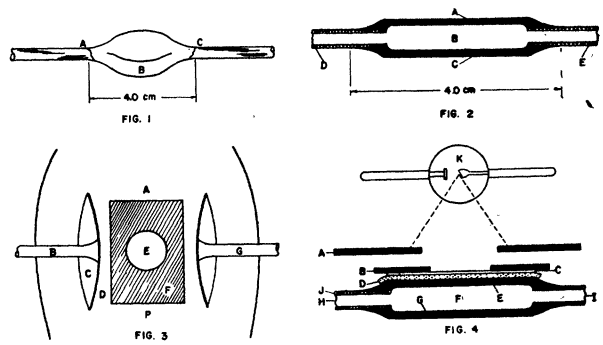

FIG. 1. Dorsolateral view of temperature-regulating chamber. *B*, chamber proper; *A, C*, junction of brass tubing inlet and outlet.

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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