days are possible without contamination. For culture identification and for protection of cover-slips the chamber is incubated in a 60 mm Petri dish, with the culture cover-slip at the bottom. Sub-cultures of cells growing in the chamber are made by trypsinization. Examples of cells examined by phase microscopy in the chamber are shown in Figs. 3 and 4; similar cells, fixed and stained, are shown in Figs. 5 and 6. For perfusion experiments, it is essential that the bottle used for collection of effluent be effectively plugged against aerial contaminants. For microscopy, the chamber is placed in a small rectangular carrier which will fit the slide clips of any microscope stage (Fig. 2). The carrier is provided with a locking screw which is useful in perfusion experiments where the position of the observed area must not change. Cell staining may be carried out either *in situ* or after removal of the cover slip on which the cells are growing, which is the method of choice.

Discussion. A tissue culture chamber is described which is simple in construction and use. It consists of 5 parts, 3 of these are standard items readily available. The parts of the chamber in contact with cells or nutrient are non-toxic. The apparatus is suitable for examination of cultivated cells at high magnifications by phase or bright-field microscopy. Cultures can be maintained for long periods of time in the chamber and subcultures are readily made. The chamber has proved valuable in tissue culture studies of material derived from cases of human leukemia, mouse leukemia, mammary carcinoma of mice, and bovine ocular squamous cell carcinoma and its benign precursor lesions.

Summary. A simple tissue culture chamber is described which permits phase microscopy at high magnifications and easy staining for permanent record. The chamber is also suited to perfusion studies, permits observation of cells over extended periods, and is useful for time lapse photography.

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Survival Time of Endotoxin Treated Rats as Response Metameter for Corticotropin and Hydrocortisone Bioassays.* (24547)

GEORGE H. GASS AND ERNEST J. UMBERGER (Introduced by A. J. Lehman)
Food and Drug Admin., U. S. Dept. of Health, Washington, D. C.

The protective effect (survival) of adrenal cortical steroids to lethal doses of typhoid endotoxin in adrenalectomized rats(1) has been suggested as the basis of assay method for adrenal cortical steroids(2), but no detailed study of the method has been published. A protective effect has been shown in normal rats, mice, and rabbits against a variety of endotoxin preparations(3,4,5) by such materials as cortisone, hydrocortisone, corticosterone, adrenal cortical extract, and corticotropin. Desoxycorticosterone acetate was without effect. Halberg, Spink, and Bittner (6) showed that cortisone, hydrocortisone, 9-α-flurohydrocortisone, and their acetates, and to some extent aldosterone, prevented hypothermia which precedes death and prolonged survival time of adrenalectomized mice treated with Brucella somatic antigen (endotoxin). We investigated the effect of hydrocortisone on hypothermia and survival time of adrenalectomized rats given a lethal dose

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of *E. coli* lipopolysaccharide (endotoxin)(7), kindly supplied to us by H. W. Schoenlein of Difco Labs. Since a large number of bioassays are available for glucocorticoids(2), it was visualized that development of an assay for hydrocortisone based on its protective effect against endotoxin in the adrenalectomized rat would provide basic information for development of an assay for corticotropin utilizing the hypophysectomized rat. Although corticosterone rather than hydrocortisone is the principal glucocorticoid elaborated by rat adrenal(8), Levitan, *et al.*(5) showed that corticosterone is effective in protecting the normal rat against a lethal dose of typhoid endotoxin. Using survival time as criterion of response, we determined dosage response curves for hydrocortisone in the adrenalectomized rat and corticotropin in the hypophysectomized rat to lethal doses of *E. coli* endotoxin.

Materials and methods. Rats of Food and Drug Administrations stock colony derived from Osborne-Mendel strain were fed standard laboratory diet and water *ad lib.* All rats were adrenalectomized or hypophysectomized the day prior to use. Following death, all animals were examined grossly for completeness of extirpation of the glands. If glandular tissue was found, the animal was discarded. Suspensions of *E. coli* lipopolysaccharide in normal saline were prepared immediately before use. Endotoxin was administered intraperitoneally in 0.25 ml containing 0.5 mg. Hydrocortisone-free alcohol was dissolved in saline and administered intraperitoneally in 0.5 or 1.0 ml. Saline was administered to control animals. Solutions of U.S.P. Reference Standard Corticotropin were prepared in saline immediately prior to each experiment. Solutions were diluted so that dose was contained in 0.5 ml and administered intravenously. *Survival time* was recorded in minutes and measured from time of endotoxin injection until expiration of animal.

Results. The effect of hydrocortisone on body temperature and length of survival of adrenalectomized rats given endotoxin was studied. Male rats weighing approximately 300 g were adrenalectomized and the following day anesthetized with pentobarbital (30 mg/kg). Thermistors were inserted rectally and body temperatures recorded each 15 minutes. Animals were divided into 3 groups of 4 animals each and injected with 0.5 mg of *E. coli* endotoxin. Immediately thereafter one group received saline, one group 25 μg hydrocortisone, and the remaining group 50 μg hydrocortisone. The results are shown in Fig. 1. It will be seen that hydrocortisone decreased the fall in body temperature and prolonged the life of rats. Preliminary experiments had shown that this amount of endotoxin caused death of adrenalectomized rats in 1 to 3 hours, a factor of practical importance. Larger amounts of endotoxin offered no further advantage and smaller amounts resulted in greater variation in the results.

There was no difference in survival time of adrenalectomized rats given a lethal dose of endotoxin when total dose of endotoxin and hydrocortisone was given as a single intraperitoneal injection or divided into 2 equal injections 30 minutes apart.

To determine the best time to inject hydrocortisone to obtain greatest protection against the lethal effect of endotoxin, hydrocortisone was administered 15 or 30 minutes before endotoxin, simultaneously with endotoxin, 15 or 30 minutes following endotoxin, and in a divided dose, one-third of dose 30 minutes before, one-third simultaneously with, and one-third 30 minutes following the endotoxin. Maximum survival time was obtained when hydrocortisone was administered simultaneously with the endotoxin or as a divided dose. Hydrocortisone given 15 or 30 minutes before or 15 or 30 minutes following the endotoxin did not increase survival time.

No statistically significant difference was noted in response to hydrocortisone between young male and female adrenalectomized rats given a lethal dose of endotoxin. However, a statistically significant difference in survival times of young and old rats was found. Young male adrenalectomized rats weighing 64 to 186 g given 0.5 mg/100 g of body weight of endotoxin survived 115.8 $\pm$ s.e. 5.6 min. while older rats weighing 224 to 453 g survived 143.6 $\pm$ s.e. 5.0 min. There was no differ-

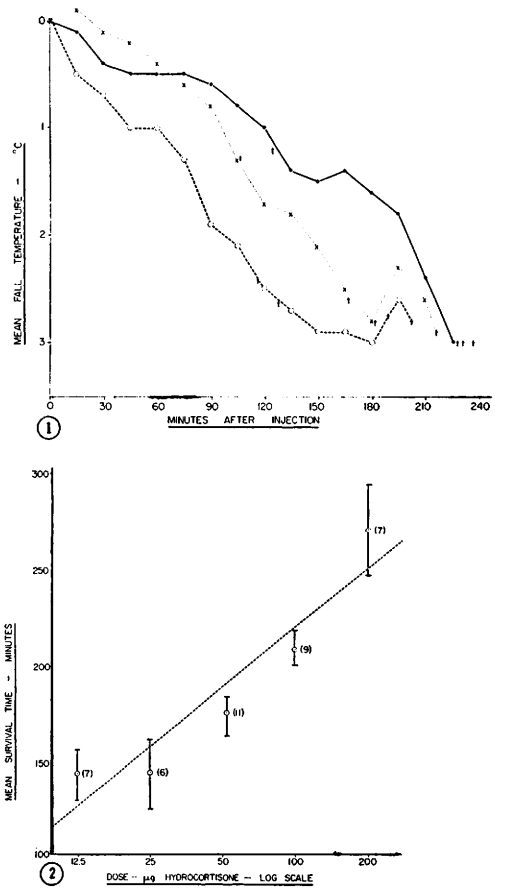

FIG. 1. Mean fall in rectal temperature and deaths (indicated by †) of male adrenalectomized rats inj. with 0.5 mg endotoxin followed immediately with saline (-○- - - -○-), 25 µg hydrocortisone (·×· · ·×·), and 50 µg hydrocortisone (-●——●-). The animals were anesthetized with 30 mg/kg of pentobarbital.

FIG. 2. Dosage-response curve for hydrocortisone in male adrenalectomized rats weighing between 150 and 220 g given 0.5 mg endotoxin immediately followed by hydrocortisone in n-saline. Figures in parentheses indicate No. of animals at each dose level and vertical lines show stand. error of mean. Equation for the line shown was calculated from all dosage levels and is $y = 9.53 + 104.83x$, $\lambda = 0.373$. For doses 12.5, 25, and 50, $y = 78.83 + 55.83x$, $\lambda = 0.390$; for doses 25, 50, and 100, $y = 5.23 + 106.97x$, $\lambda = 0.278$; for doses 50, 100, and 200, $y = -93.90 + 156.38x$, $\lambda = 0.253$. Mean survival time of 7 rats given only endotoxin and n-saline was 124.7 ± 18.0 min.

ence in response to 50 µg of hydrocortisone, survival time being increased approximately 60 minutes in both age groups.

The dose response for hydrocortisone in young adrenalectomized rat given a lethal dose of endotoxin is shown in Fig. 2. Because of limited solubility of hydrocortisone in saline, doses higher than 200 µg were not used. The curve is for all doses except the zero dose, plotting survival time in minutes as the response *vs.* log of dose of hydrocortisone in µg. Analysis of variance showed that the line was linear. It will be seen that the index of precision as indicated by λ was considerably improved by selecting higher doses. Application of Bartlett's test(9) showed that variance between responses of groups was homogeneous.

A similar dose-response relationship was determined for the hypophysectomized rat given a lethal dose of endotoxin and various doses of corticotropin. The results of 5 such experiments are shown in Table I. None of the individual lines had a statistically significant slope. However, when all the results were combined in a single assay the analysis of variance showed that the slope was significant and that the relationship was linear when the log of dose was plotted *vs.* the response (equation for line, $y = 103.90 + 37.13x$). The index of precision, λ, for the combined results was 1.069.

From the above results it appears that an assay method based on protective effect of adrenal cortical steroids to endotoxin would have an index of precision and a sensitivity comparable with other methods of assay based on stress(2). For assay of corticotropin using the hypophysectomized rat, the method appears to be less satisfactory. It is possible that in routine use of the method, a better index of precision may be obtained. We have made no studies with regard to specificity of the method.

Summary. 1. Small doses of hydrocortisone decrease the amount of hypothermia and increase survival time of adrenalectomized rats given a lethal amount of *E. coli* lipopolysaccharide (endotoxin). 2. Using survival time as the criterion of response, the dose response relationships for hydrocortisone in the adrenalectomized rat and for corticotropin in the hypophysectomized rat were determined. When the log of dose was plotted *vs.* response, the lines were linear in ranges of 10 to 200 µg of hydrocortisone and 0.5 to 4.5 milliunits of corticotropin. The indices of precision were

TABLE I. Survival Time Response of Hypophysectomized Rats Weighing 80 to 150 g Given 0.5 mg of Endotoxin Intraperitoneally Followed Immediately by Intravenous Injection of Corticotropin (ACTH).

		Total dose of corticotropin in milliunits							
		0.0		0.5		1.5		4.5	
Day	Sex	No. rats	Mean survival time, min.	No. rats	Mean survival time, min.	No. rats	Mean survival time, min.	No. rats	Mean survival time, min.
1	♂	6	92.7 ± 14.9*	5	113.8 ± 19.5*	11	145.7 ± 8.2*	9	160.8 ± 12.1*
2	♀	9	136.7 ± 10.8	7	122.7 ± 10.7	11	148.8 ± 9.1	10	140.8 ± 6.4
3	♂	9	114.4 ± 9.4	6	130.7 ± 17.5	7	105.4 ± 10.5	7	196.7 ± 31.5
4	♀	9	123.2 ± 7.7	9	157.0 ± 11.2	9	163.8 ± 11.2	11	168.7 ± 10.8
5	♂	7	125.6 ± 9.0	5	113.0 ± 10.9	6	161.8 ± 22.4	8	173.2 ± 19.1
Combined		40	120.1 ± 8.4	32	130.9 ± 6.6	44	146.0 ± 5.6	45	166.1 ± 7.2

* Stand. error of mean.

approximately 0.3 and 1.0 for hydrocortisone and corticotropin, respectively. 3. The effect of sex and age of animals and time of administration on response to hydrocortisone were studied.

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Urinary Excretion of Beta Aminoisobutyric Acid (BAIBA) in Irradiated Human Beings.* (24548)

J. R. RUBINI, E. P. CRONKITE, V. P. BOND, T. M. FLIEDNER
(With technical assistance of C. F. Dohan, Jr.)
Medical Department, Brookhaven National Laboratory, Upton, N. Y.

Recently a previously unknown amino acid, beta-aminoisobutyric acid (BAIBA) has been identified in human urines by Crumpler, Dent *et al.*(1), and Fink *et al.*(2). Further studies by Fink using C^{14} labeled thymine indicate that BAIBA is a product of thymine metabolism(3,4) (Fig. 1). Since thymine is a pyrimidine peculiar to deoxyribonucleic acid (DNA), it appears likely that under certain circumstances BAIBA excretion may reflect alterations in DNA metabolism. Aminoaciduria has been reported following total body irradiation(5,6). Analysis of the amino acids studied indicated that glycine and taurine were excreted in increased amounts from presumed protein catabolism. Pyrimidine metabolites such as BAIBA have not been studied previously to our knowledge following total body irradiation, however. Awapara(7) noted increased BAIBA excretion followed nitrogen mustard or local x-ray treatment in some leukemic patients. In June 1958, in the course of an industrial operation involving uranium, a critical geometry accidentally occurred. Eight individuals nearby were exposed to the resultant mixed neutron and

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