TABLE II. Viability of Frozen Cells Thawed in 37°C Water Bath *vs* Shortwave Diathermy.*

Slow freeze: 1-2°C/min
Storage: −170 −190°C
Medium: Eagle's, Earle's 10% calf serum
Protective additive: Glycerol 5%

Cell line	Freeze No.	Trypan blue test % viable 37°C W.B.	Trypan blue test % viable Dia-thermy	% plating efficiency 37°C W.B.	% plating efficiency Dia-thermy
HeLa	1	91†	86	40	37
Hull monkey kidney	1	78	80	16	11
	2	79	72	28	39
L cells	1	76	70	62	40
	2	76	74	45	55
Primary monkey kidney	1	57	59	25	21
Primary calf kidney	1	47	30	23	25

* Dielectric heating.
† No. represents avg of 3-8 ampules.

ampules. When plating efficiency was used to determine viability, the results averaged 48% with controlled heating vs 9, 18 and 4% with slight overheating. Extreme care must be taken, therefore, to avoid overheating when thawing.

In comparing the protective additives, it was evident that glycerol and dimethylsulfoxide were equally effective except in the primary monkey kidney freeze. In this instance, the use of dimethylsulfoxide resulted in better recoveries (Tables I, II). Viability by the trypan blue method when using DMS was 70%, with glycerol 58% and plating efficiency with DMS was 37% and with glycerol 23%.

Summary. 1. Thawing of frozen mammalian cells by well controlled shortwave diathermy is feasible. 2. Such thawing causes no apparent injury to the cells. 3. Shortwave diathermy offers no advantage over agitation in a 37°C water bath for thawing small masses of frozen tissues and cells. 4. The use of shortwave diathermy appears to be desirable for thawing large masses of tissue such as the dog or human kidney and the present studies indicate that this method can be safely used.

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Received October 11, 1963. P.S.E.B.M., 1964, v115.

Species Resistance to Histamine and Endotoxin: The Response of the Chicken.* (28940)

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Hemodynamic responses of the dog to a lethal injection of endotoxin have been well established(1). Recent studies in the monkey and observations in man have shown the pattern of endotoxin shock in the primate to differ from that in the dog(1-4). Other animals, such as the rabbit, also show unique responses to endotoxin (generalized Schwartzman reaction) not seen in most other species (5). The only consistent finding in all mammals studied (mice, rats, guinea pigs, rabbits, cats, dogs, monkeys, sheep, goats, horses, asses, and pigs) was that all succumbed to an injection of bacterial endotoxin(4,6). Conflicting evidence has been given regarding the resistance of the fowl to endotoxin and no report of hemodynamic changes has been found. Grasset *et al* have reported the mini-

* Research supported in part by Public Health Service grant.

TABLE I. Lethal Effects of Intravenously Administered Endotoxin on the Chicken.

	No. animals	mg/kg endotoxin	No. survivors
Unanesthetized	4	5*	4
	4	10	4
	5	40	5
Anesthetized	5	10	1
	1	40	0

* Two animals given endotoxin intraperitoneally; all others received intravenous injections.

mum lethal dose of crude *Bact. aertycke* endotoxin in the fowl to be 7 times that of the mouse but noted that the pigeon was apparently resistant(6). Cameron *et al,* however, found that similar endotoxin was not toxic for fowls(7). Other reports have suggested that histamine plays an important role in the development of endotoxin shock in the dog and monkey(2,8,9). The present study was undertaken to determine the hemodynamic and lethal effects of purified *E. coli* endotoxin on the chicken and to compare them with the response to histamine.

Methods. Twenty-four White Leghorn, Rhode Island Red, and Plymouth Rock chickens were used in the present study. Of these, 13 unanesthetized animals (including 2 roosters) were given 5 to 40 mg of endotoxin per kg body weight. A second group of 11 birds was studied in the anesthetized state. Sodium pentobarbital (30 mg/kg) was administered slowly through a wing vein. A catheter of PE 90 tubing (polyethylene tubing; inside diameter 0.034″, outside diameter 0.050″; obtained from Clay-Adams, Inc., New York) was inserted into the jugular vein as soon as possible since the bird regained consciousness within 10 to 15 minutes. Immediately after cannulation 2 cc of heparin (1000 U.S.P. units per cc) was given to prevent clots in the small tubing. Through this catheter additional anesthetic was given, test drugs administered, and blood samples withdrawn. A minimum volume of fluid was added. A small midline incision was made in the neck muscle and a carotid artery cannulated with PE 50 tubing (inside diameter 0.023″, outside diameter 0.038″). Arterial pressure was monitored with a Statham pressure transducer on a Sanborn recorder.

The response of the chicken to histamine was studied by administration of a range of doses of histamine acid phosphate (Lilly) and a histamine releaser, 48/80, (Burroughs Wellcome & Co.). A dose of 10 mg/kg purified *E. coli* endotoxin (Difco) was given to 5 anesthetized chickens. Additional anesthetized animals included 4 given 0.25 mg/kg 48/80, one administered 1.25 mg/kg 48/80, and one which received 40 mg/kg endotoxin. Blood pressures and heart rates were measured in all cases. Respiration rates, rectal temperatures, and hematocrits were observed in several experiments.

Results. The results from chickens given only endotoxin are summarized in Table I. All 13 unanesthetized birds survived a dose of endotoxin ranging from 5 to 40 mg/kg. Animals were considered survivors if they were alive one week after injection of endotoxin, with the exception of 2 birds in the first group given 10 mg/kg endotoxin and sacrificed after 24 hours. Five of 6 anesthetized chickens died following injection of 10 to 40 mg/kg endotoxin.

Table II lists the means and standard errors of several parameters in the 5 anesthetized chickens given 10 mg/kg of the endotoxin. The initial response was a rapid drop in arterial pressure followed by a rise to near control, a subsequent fall and finally death within 12 hours in 4 animals. Hematocrit and rectal temperature showed a decrease. In all cases, however, including 2 of the unanesthetized birds, there was an initial temperature rise followed by a progressive fall. Because of the small blood vol-

TABLE II. Effects of Endotoxin on Anesthetized Chicken.

	Systemic arterial pressure (mm Hg)	Heart rate (beats/min)	Hematocrit	Rectal temp (°C)*
Before endotoxin	110 ± 7	313 ± 11	24 ± 1	40.1 ± .3
After endotoxin (225 min)	60 ± 17	301 ± 22	17 ± 2	38.5 ± .9

* Rectal temperatures taken on 3 of the 5 chickens.

TABLE III. Effect of Endotoxin, 48/80 and Histamine on Systemic Arterial Pressure of Anesthetized Chicken.

Agent	No. exp	Magnitude of max pressure fall (mm Hg)* Mean ± S.E.	Range	Time for max fall (min)* Mean ± S.E.	Range	Time for recovery (min)* Mean ± S.E.	Range
Endotoxin	5	36 ± 8	20-65	9.6 ±9	.3-45.0 (.3- 1.3)†	37 ±22	12 -120
48/80	4	46 ± 7	25-60	.4 ± .2	.1- .7	23 ± 6	12 - 35
Histamine							
10 μg	5	29 ± 6	20-40	.2 ± .1	.1- .5	.8 ± .2	.4- .9
100 "	6	44 ± 2	35-55	.2 ± .0	.1- .3	2.2 ± .6	.7- 4.3

* Initial fall and partial recovery for endotoxin treated animals.
† Range for 4 of the 5 animals.

umes only 2 hematocrits were taken. Respiration and heart rates showed no consistent changes.

The actions of endotoxin, 48/80 and histamine are shown in Table III. The responses to these agents are compared with respect to time after injection for a maximum drop in arterial pressure, magnitude of the drop, and time required for recovery (partial recovery for endotoxin). Similarities of response with the 3 agents (using 100 μg histamine) are seen with respect to the values of maximal decrease in arterial blood pressure. Times for the maximal fall in pressure were also similar with the exception of one animal given endotoxin. Times for recoveries were distinctly different in regard to 48/80 and histamine. More sustained blood pressure depression was observed after both 48/80 and endotoxin. One animal died immediately after 1.25 mg/kg 48/80, presumably due to the direct effects of the agent. Of interest was the observation that a maximal blood pressure response to histamine occurred with a 30 μg injection; higher doses, up to 1 mg, produced only a slightly greater decrease in pressure.

Discussion. The most significant finding in the present study was that unanesthetized chickens appear to be resistant to exceedingly large doses of endotoxin. One hundred percent survival on injection of 40 mg/kg endotoxin is in marked contrast to the lethal dose of 0.55 mg/kg in the dog(4) (greater than 70-fold difference). The fact that most of the anesthetized chickens died from endotoxin could be explained by depression due to anesthesia. A steady level of anesthesia was very difficult to achieve.

Arterial pressures in both the anesthetized chicken and the dog show similar changes although hematocrits follow markedly different patterns. The chicken shows a decrease in hematocrit instead of the commonly observed hemoconcentration in the dog. The monkey also shows a decrease in hematocrit but a gradual fall in systemic pressure(2). These basic differences demonstrate the difficulties involved in extrapolation from one type of animal to another and in using only a single species in the study of the actions of endotoxin.

Although 48/80 produced nearly the same depression in arterial pressure as large doses of histamine, it required a longer recovery time. Since subsequent injections of 48/80 had little effect, it would seem that most of the available histamine was released with the first injection. There are, apparently, some direct effects due to the 48/80 itself(8), as seen in the bird given 1.25 mg/kg which died immediately. These direct actions of the drug could explain the greater response to 48/80 than to histamine.

Many similarities have been reported between the actions of histamine and endotoxin in the dog(8). The release of histamine after endotoxin has been observed in the dog and monkey(2,9). With the notable exception of one animal, the birds given endotoxin show similar responses to those receiving 48/80 and histamine. It is possible, therefore, that the initial fall of systemic pressure after endotoxin in the chicken is due to a release of

histamine. Its failure to show a greater drop in pressure to large doses of histamine may be a factor involved in the resistance of the unanesthetized chicken to endotoxin.

Summary. Hemodynamic and lethal effects of endotoxin in the chicken were studied. It was noted that the unanesthetized bird is highly resistant to endotoxin, all animals surviving intravenous doses up to 40 mg/kg. Systemic arterial hypotension and a slight degree of hemodilution were observed in the anesthetized chicken given 10 mg/kg endotoxin. The possible role of histamine is discussed.

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Received October 14, 1963. P.S.E.B.M., 1964, v115.

Respiratory Syncytial Virus: Properties of Strains Propagated in Monkey Kidney Cell Cultures.* (28941)

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Respiratory syncytial (RS) virus is unstable, undergoing rapid inactivation under usual conditions for maintaining viruses. Inactivation can be slowed by storage of virus stocks in chicken sera, or in gelatin, but with these, decay continues, although at reduced rates. During recent studies on respiratory diseases we recovered 24 RS viruses from 18 infants and children. During our work we found that RS viruses may be preserved in 50% glycerin for at least 9 months.

Another aspect of the study deserves mention. During the outbreak in the spring of 1962 RS viruses were detected more often in cultures of monkey kidney than in other cell lines. Although it has been known that monkey kidney cell cultures support the growth of RS virus, several writers(1-3) have found these cells less satisfactory than those of human origin. Our studies subsequently led us to define several properties of RS viruses propagated in monkey kidney cell cultures.

* This investigation was supported in part by U.S.P.H.S. research grant from the Nat. Cancer Inst.

Materials and methods. Strains of virus. Procedures used for isolating these viruses have been described(4). "Long" strain of RS virus was received from Chanock in 1960. This strain has been propagated 18 times in HEp-2 cells, and 20 to 25 times in monkey kidney cell cultures in our laboratory. The "87" strain of RS virus which was isolated during the 1962 studies was passed 20 to 25 times in monkey kidney cell cultures. Infectivity titers for both strains were 10^{-5} to $10^{-5.5}$ $TCID_{50}$ per ml when titrated in monkey kidney cell cultures.

Growth curves. Monkey kidney cells were grown in monolayers in tubes; cells were inoculated either with "Long" or "87" strains of RS virus. The input of infectious virus, using undiluted stocks, was such that about every hundredth cell became infected. Cultures were incubated at 37°C on a "roller drum." Three hours after inoculation the fluid overlay was drawn, the cell sheet was washed twice with PBS and fresh medium was put on the cell sheet. Fluid overlays from 4 tubes were collected thereafter at