Effects of Hydrocortisone on Survival in Neonatal Beagles Given Endotoxin* (33007)

JENNIFER M. H. LOGGIE, PHILIP J. PRIVITERA, SIGMUND SUGARMAN,[1] AND THOMAS E. GAFFNEY[2]

Division of Clinical Pharmacology, Departments of Pharmacology, Pediatrics and Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

Hydrocortisone, given either before or after an otherwise lethal intravenous dose of bacterial endotoxin, has been shown to increase the survival rate of adult dogs (1,2). As a consequence of the improved survival with corticosteroids in mature animals, hydrocortisone (HC) has been advocated and is widely used in the treatment of human septic shock.

The human neonate is particularly susceptible to infection with gram-negative organisms but the pathophysiology of septic shock in this age group has not been studied. Similarly, neither the pathophysiology nor the therapeutic efficacy of various drugs have been extensively studied in the neonatal laboratory animal.

Reddin *et al.* (3) have recently shown that the cardiovascular and humoral responses to intravenous bacterial endotoxin (ET) in the mongrel puppy differ from those observed in adult dogs. It has also been shown that there are differences between adult and newborn guinea pigs in both the fever and leucopenia produced by ET administration (4). Because of these differences and because it is known that drug effects are unpredictable in young animals, we have studied the effect of HC, given both before and after ET, on survival in young beagles given *E. coli* ET. There are data on the effects on survival of corticosteroids given simultaneously with ET to the embryo chick (5) and before ET, to the neonatal guinea pig (4). There are however, no data on the effects of corticosteroids given after ET to the newborn of any species. In view of the fact that in the clinical situation HC is given after the development of septic shock, it seemed particularly important to test its efficacy in a similar situation in the neonatal laboratory animal.

Methods. Six- to 9-day-old pure bred beagle puppies (weight 240–750 gm) were anesthetized with pentobarbital 15 mg/kg i.p. and a polyethylene cannula was inserted into the left femoral vein for the administration of ET, HC, and saline. An aseptic technique was not used nor were antibiotics given.

Body temperature was maintained between 37–39°C (rectally) and a clear airway was ensured by frequent application of suction. Animals were not starved before the experiments and survivors were fed by naso–gastric tube 8 hours after ET and at 4–6 hourly intervals thereafter.

The intravenous dose of ET that was lethal to untreated animals within 8 hours was established at 2 mg/kg (Difco *E. coli* endotoxin, batch 484867). Two animals received only pentobarbital 15 mg/kg i.p. and in three animals, pentobarbital anesthesia and catheterization of the femoral vein were the only procedures. All five puppies survived these procedures. In addition, three animals received HC 150 mg/kg i.v. without ET and survived without apparent ill effects.

Four types of experiment were performed; in the first three types, animals were paired for weight, sex, and litter, and HC or saline were administered in a double blind, randomized fashion after ET. All dogs which lived 72 hours or more were considered to be permanent survivors.

Group I. 14 animals received either hydrocortisone sodium succinate (Solucortef[3]) 50 mg/kg, or saline, immediately after a single dose of ET. The dose of ET ranged between 0.1 and 2.0 mg/kg.

* The research reported in this paper was supported by the Life Insurance Medical Research Fund, and USPHS grants HE07392 and HE05622.

[1] Medical student; Fellow in Clinical Pharmacology July–Sept. 1966.

[2] Research Career Development Awardee; National Health Institute; USPHS.

[3] Upjohn Company, Kalamazoo, Michigan.

Group II. Four doses of ET were given at 30-min intervals to 12 dogs. The total dose of ET ranged between 0.4 and 1.6 mg/kg. The HC 50 mg/kg or saline was given 10 min after the initial dose of ET.

Group III. The ET was given in 2 doses, separated by a 30-min interval, to 12 beagles. The total dose varied between 0.2 and 2 mg/kg. The HC 50 mg/kg or saline was given 10 min after the first dose of ET.

Experiments in Groups II and III were designed to simulate the situation in clinical septic shock where ET is believed to be repeatedly released into the circulation.

Group IV. The HC in a dose ranging from 5 mg/kg to 150 mg/kg was given to 25 animals 1 hour prior to the administration of ET 2 mg/kg. The doses of HC were 5 mg/kg in 5 animals; 15 mg/kg in 5 animals; 50 mg/kg in 9 animals; and 150 mg/kg in 6 animals. Fourteen animals given saline 1 hour before ET served as controls.

Results. Group I. (HC after a single dose of ET.) There was no difference in survival rate or survival time between animals which received HC as opposed to saline. At the 0.1 mg/kg dose of ET both the HC- and saline-treated dogs survived permanently. Apart from this pair, all dogs (0.5 and 2 mg/kg) were dead within 8 hours.

Groups II and III. (HC after the first of 4 or 2 doses of ET.) In neither of these groups

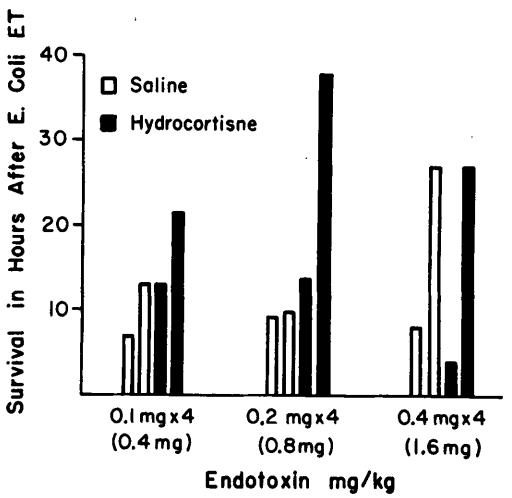

FIG. 1. Effect of HC 50 mg/kg on survival when given 10 min after first of 4 doses of ET.

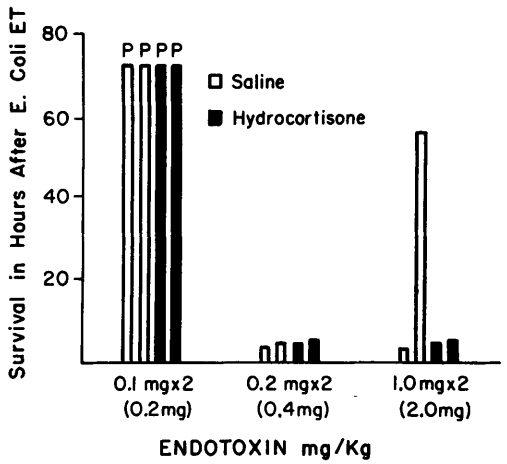

FIG. 2. Effect of HC 50 mg/kg on survival when given 10 min after first of 2 doses of ET.

was there a significant increase in survival rate in HC-treated animals (Figs. 1 and 2). The four animals given 0.1 mg/kg of ET in 2 doses all survived permanently irrespective of whether they received HC or saline. There were no other permanent survivors. Two HC-treated animals in Group II had an increased survival time compared to their saline-treated counterparts. It should be pointed out that survival time did not appear to be decreased by treatment with HC in any of the animals in Groups I, II, and III.

Those animals which received HC after ET developed bloody–mucoid diarrhea, became progressively pale, and had rapid, grunting irregular respirations. The picture they presented was no different from that seen in saline-treated control animals. At autopsy there was no gross difference between the organs of HC-treated as compared to saline-treated animals. All showed intense congestion of the liver, the small and large intestine, and sometimes the stomach. Most animals had markedly congested kidneys with petechial hemorrhages on the surfaces, and the adrenals were in many instances congested.

Group IV. (HC pretreatment, 5–150 mg/kg) All 14 control animals pretreated with saline died within 7 hours of receiving ET. In contrast, HC pretreatment increased survival time in some of the animals at all dose levels tested (Fig. 3). An increase in survival rate was also observed at three of the four dose

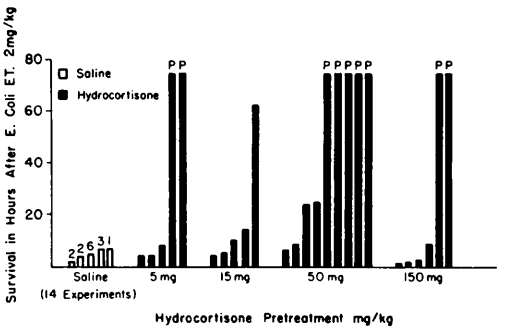

FIG. 3. Effect of HC 5-150 mg/kg on survival when given 1 hour prior to *E. coli* ET 2 mg/kg.

levels tested. None of these three doses of HC was superior to the others in terms of survival rate: 2 of 5 animals survived permanently at the 5 mg/kg dose, 5 of 9 at the 50 mg/kg dose and 2 of 6 at the 150 mg/kg dose of HC.

No animal pretreated with HC was autopsied. Most of these animals appeared less ill after ET than their saline-treated counterparts, although they all developed profuse bloody diarrhea, which lasted up to 48 hours.

Discussion. In 1962 Uhr (4) showed that the neonatal guinea pig is more susceptible to the lethal effects of intravenously administered bacterial ET when compared with the adult of the same species. He also showed that pretreatment of newborn guinea pigs with either 5 mg of cortisone or 5 mg of HC i.m. increased the rate of survival. He did not attribute the sensitivity of the newborn animals to ET to hypofunction of the adrenals, since pretreatment with cortisone provided only partial protection.

Smith and Thomas (5) have shown that the chick embryo is partially protected from the lethal effects of either *E. coli* or meningococcal ET when either is given simultaneously with cortisone, HC, or 9-alpha-fluorohydrocortisone. The chick embryo is particularly susceptible to the lethal effects of ET at 10 days of age when the adrenal medulla is differentiated and capable of epinephrine synthesis but when the adrenal cortex is not yet functional. This evidence suggests that in the chick embryo at the time of maximum sensitivity to ET there is adrenocortical insufficiency.

Our data indicate that the 1-week-old puppy is susceptible to the lethal effects of ET and can be partially protected from this lethal effect only if HC is given before ET. These findings are in contrast to those of Lillehei and Weil (2) who found that treatment of adult dogs with glucocorticoids after ET increased both the survival rate and survival time.

The mechanism of protection by HC in animals treated prior to the administration of ET is not known. There is evidence that neither the adult dog (6), nor adult man (7), suffers from adrenocortical insufficiency during endotoxin shock. There is also evidence (8) that children (1 month–16 years of age) with various forms of meningitis have increased levels of plasma cortisol except in the presence of adrenal hemorrhage. The normal human neonate is deficient neither in adrenocortical (9) nor adrenomedullary (10) hormones; and in the animals in this study, in which agonal plasma catecholamines were measured, high values for both norepinephrine and epinephrine were obtained. No attempt was made to measure plasma cortisol levels; it is possible that the newborn, unlike the adult, may develop a relative adrenocortical insufficiency under severe or prolonged stress.

The failure of HC to protect young animals when given after even small doses of ET has not previously been reported. This observation suggests that the human neonate in septic shock would not be benefited by the administration of large doses of HC. It is possible that HC acts at a cellular level to protect tissues from damage by ET and that when ET induced cellular injury already exists this protective effect of HC is lost.

The importance of the relationship between the concentration in the plasma of readily diffusible cortisol and the degree of tissue damage has been demonstrated by Melby *et al.* (6). When large doses of cortisol were administered with ET to adult dogs there was a smaller rise in transaminase levels than when ET was given alone. It is known that hepatic enzyme function is immature in the human neonate and it is possible that the same immaturity exists in the young dog. In the newborn dog given ET there may be sufficient hepatic dysfunction to impair the young animals' ability to split off the sodium suc-

cinate ester of HC, thereby preventing the release of free, active HC.

Summary. In beagles 6–9 days old, hydrocortisone sodium succinate (5–150 mg/kg), given intravenously 1 hour before intravenous lethal doses (2 mg/kg) of *E. coli* endotoxin, increased the rate of survival, and, in some animals, the duration of survival; the effect of hydrocortisone was not dependent on the dose given. Hydrocortisone (50 mg/kg) given immediately after endotoxin (0.1–2 mg/kg) did not increase the rate or duration of survival.

1. Lillehei, R. C., Longerbeam, J. K., Block, J. H., and Manax, W. G., Ann. Surg. **160**, 682 (1964).

2. Weil, M. H. and Miller, B. S., J. Lab. Clin. Med. **57**, 683 (1961).

3. Reddin, J. L., Starzecki, B., and Spink, W. W., Am. J. Physiol. **210**, 540 (1966).

4. Uhr, J. W., J. Exptl. Med. **1185**, 685 (1962).

5. Smith, R. T. and Thomas, L., J. Exptl. Med. **104**, 217 (1956).

6. Melby, J. C., Egdahl, R. H., and Spink, W. W., J. Lab. Clin. Med. **56**, 50 (1960).

7. Melby, J. C. and Spink, W. W., J. Clin. Invest. **37**, 1791 (1958).

8. Migeon, C. J., Kenny, F. M., Hung, W., and Voorhess, M. L., Pediatrics **40**, 1963 (1967).

9. Migeon, C. J., Green, O. C., and Eckert, J. P., Metab., Clin. Exptl. **12**, 718 (1963).

10. Voorhess, M. L., Pediatrics **39**, 252 (1967).

Received Jan. 11, 1968. P.S.E.B.M., 1968, Vol. 128.