Effect of 3, 4-Dichloroisoproterenol (DCI) on Body Temperature Changes Induced by Bacterial Endotoxin. (26499)

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It is known that peripheral vasoconstriction occurs coincident with the onset of the pyrogenic reaction. Wells and Rall(1) suggested that the rise in body temperature resulted from a sudden and marked reduction in heat loss. They were later able to reduce and alter the febrile response in dogs with an adrenergic blocking drug(2). They induced fever in normal and in curarized dogs with intravenous doses of pyrogen obtained from *Pseudomonas aeruginosa*. N-ethyl-N-(2-bromoethyl)-1-naphthylenemethylamine · HBr was their blocking agent. This compound is similar in mode of action to phenoxybenzamine. It blocks the alpha receptors according to the hypothesis of Ahlquist (3). A peripheral vasoconstriction, mediated by excitatory adrenergic responses in the skin, is regarded by Wells and Rall as the means by which heat loss is reduced and body temperature is raised. Kroneberg and Kurbjuweit(4) were able to inhibit bacterial pyrogen-induced fever in rabbits by pretreating the rabbits with reserpine. Iproniazid opposed the reserpine effect. This constitutes additional evidence that adrenergic mechanisms are involved but does not distinguish between excitatory or inhibitory factors.

The compound DCI or 3,4-dichloroisoproterenol is considered to be a specific blocker for inhibitory adrenergic responses(5,6). It was the only such drug which offered any protection against bacterial endotoxin described previously(7). Therefore, it was tested for ability to oppose the pyrogenic effects of *E. coli* endotoxin in unanesthetized rabbits.

Methods. Rectal temperature was measured with a thermistor in normal unanesthetized white rabbits restrained in an open stock. A "fever index" was calculated in the manner of Beeson(8) by taking as a base level the animals' temperature at time of injecting the pyrogen, and measuring with a planimeter, the area enclosed between this line and the course of the elevated temperature. When temperature failed to return to the base level within the time limit a vertical line was drawn between the last temperature and the baseline. Fig. 1 illustrates this method. DCI and phenoxybenzamine solutions were made up in pyrogen-free injectable saline which was also used for the saline controls. These pretreatments were given to the rabbits subcutaneously 24 hours and 1 hour before endotoxin challenge. Difco crystalline lipopolysaccharide from *E. coli* (Code 0127:B8, Control 118023) was the endotoxin. It was also dissolved in pyrogen-free saline and given intravenously in the marginal vein of the ear. Similar observations were made in mice with endotoxin being given intraperitoneally.

Results. The results of these tests are summarized in Table I. Very little, if any, protection is apparent. The maximum rise in body temperature is the same in controls and DCI pretreated rabbits after 1.0 μg/kg i.v. endotoxin. At a dose of 5 μg/kg the control maximum was 0.4°F less than that of the pretreated rabbits but at 7 μg/kg, the opposite effect was seen. The DCI pretreated rabbits all had substantial fevers. In contrast, a group of rabbits pretreated in similar fashion with phenoxybenzamine experienced a very slight rise in body temperature.

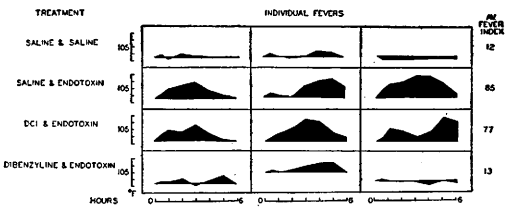

FIG. 1. Fevers in rabbits presented in the graphic manner of Beeson (1947). Degrees Fahrenheit are plotted on ordinates against time in hr on abscissae. Indices on right are obtained with a planimeter. 'Dibenzyline' is the trade name for phenoxybenzamine.

TABLE I. Average Rectal Temperatures of Groups of Rabbits in Degrees Fahrenheit.

No. of rabbits	Pretreatment Drug	Dose, mg/kg s.c.	Endotoxin dose, i.v. μg/kg	Control	Test intervals after endotoxin in hr .5	1.0	2.0	3.0	4.0	5.0	6.0	Maximum effect
6	Saline blanks	—	(Saline)	103.7	103.4	103.3	103.4	103.4	103.6	103.6	103.5	− .4
2	" "	—	1	104.3	105.5	106.0	105.9	107.1	106.8	105.8	105.2	+2.8
3	DCI	2 × 10	1	103.8	104.7	105.2	105.2	106.6	106.6	105.6	105.7	+2.8
5	Saline blanks	—	5	103.3	103.9	104.3	104.5	105.7	105.7	105.0	104.1	+2.4
5	DCI	2 × 10	5	103.7	104.5	105.1	105.3	106.5	106.2	105.3	104.5	+2.8
3	Phenoxybenzamine	2 × 3	5	104.1	104.3	104.2	104.5	104.3	104.6	105.1	104.1	+1.0
3	Saline blanks	—	7	104.0	105.3	106.2	106.7	107.4	107.6	106.9	105.7	+3.6
3	DCI	2 × 10	7	103.5	104.2	104.6	105.4	106.2	106.1	105.2	104.0	+2.7

The effects of pretreatment with DCI and with phenoxybenzamine are also shown in Fig. 1. The first tier of areas are controls. Below this the fevers produced in 3 rabbits by an intravenous dose of 5 μg/kg of endotoxin are portrayed. In order beneath these are similar responses in 3 rabbits pretreated with 2 doses of DCI 10 mg/kg, given 24 hr and 1 hr before the endotoxin. The lower tier clearly shows the antipyretic effect of phenoxybenzamine. This was to be expected from the report by Wells and Rall. Although the fever index is somewhat lower in the DCI pretreated animals than in those that received saline before endotoxin this difference is negligible when compared with the phenoxybenzamine effect. Similar comparisons were made between saline and DCI pretreated rabbits at doses of 1 and 7 μg/kg i.v. endotoxin. The fever indices in the first case were 62 (saline) *vs.* 63 (DCI) and 102 *vs.* 84 in the second. Although fever indices in the DCI pretreated rabbits were lower at both the 5 μg/kg and 7 μg/kg doses of endotoxin and a slight protective effect may be suggested it is not considered adequate to explain previous findings(7). Here phenoxybenzamine failed to offer protection against the lethal effect of bacterial endotoxin while DCI was clearly beneficial. In these rabbit tests phenoxybenzamine was markedly antipyretic and DCI was not.

Berry, Smythe and Young(9) had noted hypopyrexia rather than fever when large doses of endotoxin were given to mice. It seemed possible that their effects might be the result of heat loss through vasodilation. Such an effect would be opposite that proposed by Wells and Rall. If so, it might be susceptible to inhibition by DCI. To investigate this possibility 2 series of body temperature measurements were made in mice treated and untreated before endotoxin administration. These are presented in Fig. 2. Although average temperatures for the saline control

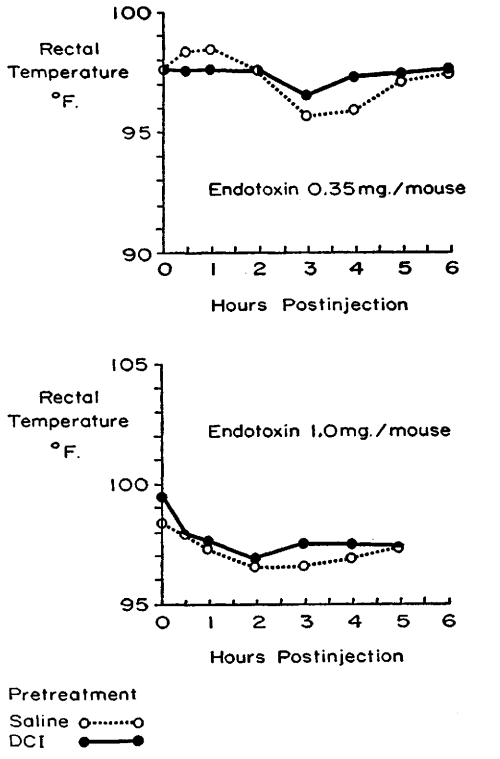

FIG. 2. Rectal temperatures of mice after intraper. inj. of 0.35 or 1.0 mg *E. coli* endotoxin. Each point is the avg of at least 10 determinations. DCI dose is 2 × 10 mg/kg s.c. (24 hr and 1 hr) before the endotoxin.

groups were slightly lower than comparable figures for DCI pretreated animals statistical treatment indicated that the apparent differences were not at all significant.

It would appear from this series of experiments that the unique type of adrenergic blockade produced by DCI(5,6) is not effective against either fever or heat loss. It is concluded that some mechanism other than body temperature is concerned in the protective action of DCI which has been observed in mice challenged with *E. coli* endotoxin(7).

These data also suggest that the pyrogenicity of an endotoxin is not directly correlated with its toxicity. This is an important observation since many investigators use as an assay for endotoxin its ability to produce hyperthermia in experimental test animals. If the assay employed fails to measure the originally significant property of an endotoxin, *i.e.*, its toxicity, then the value of the assay needs thoughtful evaluation.

Summary. 1) Pretreatment of rabbits with DCI failed to prevent the pyrogenic reaction to injected bacterial endotoxin. 2) The hypopyrexia produced in mice by endotoxin likewise was unaffected. 3) It is concluded that the type of adrenergic inhibition produced by this substance is not germane to the problem of body temperature changes induced by bacterial endotoxins.

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Received January 11, 1961. P.S.E.B.M., 1961, v106.

Partial Replacement of Serum and Embryo Extract Requirements for Growth of Avian Cell Cultures.* (26500)

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Embryo extract has been employed extensively in tissue culture(1) since the discovery of its stimulatory effects by Carrel(2). Modern developments have eliminated this material in a majority of instances from the requirements of mammalian cells(3); nevertheless, avian cultures, upon which the initial observations were made, have continued to require embryo extract or components of embryo extract for satisfactory growth(4,5,6). Serum, traditionally necessary for mammalian cells, also has been essential to avian cultures in addition to the embryo extract(4,5,6).

The present report concerns the growth response of avian cultures to constituents of a serumless medium devised for cultivation of mammalian cells(7).

Methods and materials have been described previously(6). In brief, cell suspensions of 14-16 day white Leghorn chick embryo lung or heart were prepared by stirring the minced tissue 2-4 hours with 0.2% Difco 1:250 trypsin in Earle's balanced salt solution at 37°C. The cell suspension filtered through gauze and collected by centrifugation was resuspended in a complete medium containing serum and embryo extract(6). One ml amounts of the suspension containing 50,000 to 100,000 cells per ml were pipetted into 15 × 125 mm roller tubes placed in a slanted position in racks. The cultures were established

* This work was completed under contract with Cancer Chemotherapy National Service Center, Nat. Cancer Inst., N.I.H., U.S.P.H.S.