Study of the Effect of Hematoporphyrin on Hypophyseal-Adrenal Relationships.* (20328)

GEORGE C. PECK, H. PATTERSON MACK, AND FRANK H. J. FIGGE.

From the Department of Anatomy, University of Maryland, School of Medicine, Baltimore.

It had been shown(1) that porphyrins injected into ovariectomized mice produced a series of estrous reactions, which reoccurred after the porphyrin injections were discontinued. It had also been shown that injection of porphyrins into amphibians caused a release of the melanophore-expanding hormone from the intermediate lobe of the hypophysis(2). It was, therefore, concluded that porphyrins had a stimulatory effect on the hypophysis and that the reactions following porphyrin injections were related to the secondary stimulation of the adrenal cortex, gonads, or melanophore system(3). When one of us attempted to induce estrous reactions by injecting porphyrin into ovariectomized mice, vaginal smears indicative of pseudo-estrous were obtained, but the results were not considered conclusive. Other methods to test the possible stimulatory influence of porphyrins on the hypophysis were then devised. In order to study any potential hypophyseal stimulator, the degree of specificity of response of the hypophysis should be evaluated. At the time that ACTH and Cortisone became popular therapeutic agents, it seemed desirable to devise a method of stimulating the hypophysis to produce greater amounts of ACTH, and it was hoped that hematoporphyrin would give rise to such specific stimulation.

The hypothesis involved in these experiments was also based on the observations of Nelson(4), who demonstrated that Cortisone had a definite inhibitory effect on anaphylaxis in the mouse. Since ACTH stimulates the adrenal cortex to elaborate Cortisone and Cortisone-like compounds, it was postulated that if porphyrin stimulated hypophyseal ACTH output, it might thus have an indirect Cortisone-like influence on anaphylaxis in the mouse. These experiments, therefore, involved studies on the influence of porphyrins, ACTH, Cortisone, and stress on anaphylaxis in the mouse. It will be shown that Cortisone did indeed prevent death from anaphylactic shock in the mouse(4) and that high doses of porphyrin also protected mice from anaphylactic shock. ACTH and low doses of porphyrin and various forms of stress either had no protective effect or increased the incidence of death from shocking doses of horse serum.

Methods and materials. The 446 mice used in these experiments were of the LCS_a,† Strong A, and C_3H strains. They were sensitized by injecting 1 cc of neutral undiluted horse serum‡ intraperitoneally every second day until a total of 4 cc had been administered to each mouse. This procedure is essentially the same as the method used by Mayer and Brousseau(5). After a sensitization period of 2 weeks to 2 months, each mouse received 1 cc of the same antigen by a rapid injection into the tail vein. The groups of mice were divided into control and experimental groups. The controls received no injections in the interim prior to the shocking dose of horse serum, except a few groups that received injections of physiological saline. Mice in the experimental groups received either hematoporphyrin, aqueous ACTH, Cortisone, or stress treatment 4-48 hours before the shocking dose of horse serum. The hematoporphyrin was given intraperitoneally; the ACTH and Cortisone intramuscularly. The dosages given to the various groups and the results are indicated in Table I. The 4 and 6 mg total doses of hematoporphyrin were divided into individual doses of 2 mg each, administered 24 and 4 or 48, 24, and 4 hours before the administration of a shocking dose of horse serum. When a sensitized control mouse was given 1 cc of horse serum by tail vein injection

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† The LCS_a strain was developed in this laboratory by crossing a CBAN with the Strong A mouse.

‡ Wyeth (without preservative)

TABLE I. Influence of Various Agents and Procedures on Survival of Sensitized Mice Injected with Shocking Doses of Horse Serum.

Drug inj.	Dose, mg	No. mice	No. survived	% survived	Difference from control	S.E., %	Difference/ S.E. of difference
Controls		148	38	26		3.6	
Hematoporphyrin	.6– 2	45	3	6.6	—20	3.7	3.9
"	4 – 6	86	39	45.3	+19	5.4	2.9
ACTH	2 –10	89	25	28	+ 2	4.7	.3
Cortisone	3	41	41	100	+74	0	20
Stress							
Phenol		19	0	0	—26	0	7.2
Air jet		18	1	5.5	—21	5.1	3.3

after a normal sensitizing period, the mouse usually died. The criterion for a protective effect of any treatment was simply based on whether a significantly greater percentage of the mice survived such a shocking dose of horse serum.

Observations and results. The number of mice in each group and the treatment received are shown in Table I. This also gives the number of mice that survived such treatment and the percentage of survival along with the standard error and an estimate of the significance of the differences observed. In all, 148 control mice were sensitized, and 38 of these survived a shocking dose of 1 cc of horse serum. This is a 26% survival. Forty-five mice were injected with low dosages of hematoporphyrin (0.6 mg to 2 mg). Only 3 of these survived the shocking dose of horse serum. This is a 6% survival. The difference between this and the control group was fairly large (20%) and appeared to be statistically significant.

When mice were injected with 4-6 mg of hematoporphyrin in divided doses 2 days before they received the shocking dose, a total of 39 (45%) of the 86 mice survived. This differs from the control group by 19% and involved a relatively large number of animals. Since this difference was 2.9 times the standard error of the difference, it is felt that high doses of porphyrin exerted a protective influence. The per cent survival of the mice receiving 4 and 6 mg of hematoporphyrin were so nearly the same that they were grouped together.

Eighty-nine of the sensitized mice were injected with aqueous ACTH (2-10 mg); one half of the dose was given 24 hours; the other half 4-6 hours before the administration of a shocking dose of horse serum. Twenty-five of the 89 mice or 28% survived. This is only 2% more survivors than in the control group. It was concluded that ACTH did not protect mice from anaphylactic shock.

In contrast, the protective effect of Cortisone was most striking. Forty-one of the sensitized mice were injected with 3 mg of Cortisone 20 hours before the intravenous injection of horse serum. All of the 41 mice survived. The difference between that and the control group was 74% or 20 times the standard error of the difference and is, therefore, highly significant.

As indicated in the table, 2 types of stress were used in the attempt to evaluate the influence of stress reactions on anaphylactic shock. Nineteen of the mice were injected with 0.5 cc and 0.25 cc of 0.5% phenol 24 and 4 hours before the shocking dose of horse serum. None of these mice survived. Eighteen of the mice were subjected to an air jet for 2 minutes, 24 and 4 hours prior to the injection of a shocking dose of horse serum. Only 1 mouse survived. At the time when the shocking dose was administered, the physical appearance and behavior of the mice in both the phenol-treated and air jet-stressed groups was normal. Neither of these procedures protected mice from anaphylactic shock, but on the contrary, had an adverse effect which was found to be statistically significant.

In studying the effect of stress or alarm reactions on anaphylactic shock in egg-albumin-sensitized guinea pigs, Leger, Leith, and Rose(6) found that mild stress protected the animals from anaphylactic shock. However, if severe stress and alarm reactions were

elicited, and a shocking dose of egg albumin was given a few hours later, but before the alarm reaction had subsided, it actually aggravated the anaphylaxis. This may possibly explain some of our low survivals in the group of mice stressed with phenol injections and air jets.

Summary and conclusions. 1. Four hundred and forty-six mice were sensitized to horse serum, and 295 of these were treated with ACTH, Cortisone, hematoporphyrin, phenol, and air jets, in order to compare their influence on the survival of these mice when given a shocking dose of horse serum. 2. Twenty-six percent or 38 of 148 control mice sensitized to horse serum survived. 3. One hundred percent (41) of the horse serum-sensitized mice survived a shocking dose when treated with 3 mg of Cortisone 24 hours prior to the injection of 1 cc of horse serum. 4. ACTH failed to protect sensitized mice from shocking doses of horse serum, as did low doses of hematoporphyrin and stress procedures. Both of the latter procedures appeared to increase the incidence of death from anaphylaxis. 5. Hematoporphyrin in doses of 4-6 mg protected mice from shocking doses of horse serum, since a significantly higher percentage (45%) of the mice survived. 6. While our evidence indicates that high doses of hematoporphyrin protect mice from anaphylactic shock, no conclusions can be drawn concerning the hypothesis that porphyrins stimulate the hypophysis, because ACTH failed to exhibit any influence on anaphylaxis.

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Effects of X-Irradiation and Cysteine Injection on Enzyme Systems of Rat Tissues.* (20329)

MARIE A. FISCHER, ELLEN PURVIS COULTER, AND MICHAEL J. COSTELLO.
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From the Department of Physiological Chemistry, School of Medicine, University of Pittsburgh.

Barron and his co-workers(1) reported that purified sulfhydryl-containing enzymes are inactivated by small amounts of x-rays and that the activities can partially be restored by glutathione. In the rat the oxidations of substrates requiring sulfhydryl-enzymes were shown to be diminished in all tissues, except the thymus, as a result of whole body x-irradiation(2) and it was demonstrated by Patt, Tyree, Staube and Smith(3) that cysteine or glutathione, administered prior to exposure, increased the number of animals able to survive a lethal dose of x-rays. Ludewig and Chanutin(4) found no change in the activity of rat liver catalase and certain other non-thiol enzymes but Feinstein, Butler and Hendley(5) demonstrated that a reduction in liver catalase activity follows the exposure of the whole mouse to x-rays. Barron(2) found that the oxidation of added succinate by kidney slices was depressed as a result of x-ray treatment, but LeMay(6) failed to find a decrease in the succinoxidase or cytochrome oxidase activities of kidney homogenates or slices. Citrate synthesis in the spleen, thymus, ileum, pancreas and testes of the rat is depressed by lethal doses of x-ray(7) but the activities of succinoxidase, adenosinetriphosphatase, and other sulfhydryl enzymes of several tissues were unaffected. More recently Ashwell and Hickman(8) showed that whole body irradiation does not affect spleen

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