during infusions of graded doses of isoproterenol. The increments in plasma FFA during isoproterenol administration were significantly attenuated by propranolol in the hypermetabolic period as well as during the initial and final control periods. Neither isoproterenol nor propranolol produced any appreciable changes in blood glucose in any period. The results also suggest that beta adrenergic receptors may mediate the effects of isoproterenol upon free fatty acid mobilization but do not suggest a similar receptor role for regulation of blood sugar levels.

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Selective Suppression of Penicillin Hypersensitivity by Certain Phenoxy Propanediols. (31728)

F. M. BERGER, G. FUKUI, B. J. LUDWIG, AND S. MARGOLIN
Wallace Laboratories, Cranbury, N. J.

Little work has been reported on the broad concept of interference with immunological reactions by simple chemical compounds. It has been known for some time that certain simple dyes may specifically enhance or suppress the reaction between the syphilis reagin and the corresponding antibody(1). More recently it was shown that peritoneal mononuclear phagocytes obtained from guinea pigs that have been treated with meprobamate do not support the intracellular growth of *Brucella abortus* and thus behave like cells obtained from immunized animals(7). It was of interest to see whether meprobamate would also be able to affect certain other immune responses. The passive cutaneous anaphylaxis reaction (PCA) elicited in the guinea pig with penicillin conjugates and bovine serum albumin (BSA) was selected as the experimental model. Meprobamate did not interfere with this reaction. However, certain phenoxy propanediols selectively inhibited the penicillin induced anaphylaxis without affecting anaphylaxis induced by other proteins. This interesting observation was followed up not only because of theo-

TABLE I. Effect of Chlorphenesin on PCA Reactions Induced by Penicillin G (KPG) or Bovine Serum Albumin (BSA).

Treatment given intraperitoneally 30 min prior to antigen	Guinea pig No.	Antisera dilutions: Anti-KPG 1/25	Anti-KPG 1/50	Anti-KPG 1/100	Anti-BSA 1/2000	Saline
Saline (control)	1	3+	2+	0	4+	0
	2	3+	2+	1+	4+	0
	3	3+	3+	2+	4+	0
	4	3+	3+	3+	4+	0
	5	2+	0	0	4+	0
Chlorphenesin, 100 mg/kg	6	0	0	0	4+	0
	7	0	0	0	4+	0
	8	0	0	0	4+	0
	9	0	0	0	4+	0
	10	0	0	0	4+	0

retical implications but also because of the potential clinical importance of a drug that would prevent or counteract the so prevalent penicillin hypersensitivity reactions.

Materials and methods. The passive cutaneous anaphylaxis test (PCA) has been performed according to the method of Ovary (13). Male Hartley strain guinea pigs weighing 250 to 350 g were used. After the hair had been removed from the back with an electric clipper, serial dilutions of antisera were injected intracutaneously in a volume of approximately 0.1 ml. As a rule, three dilutions of penicillin antiserum, 2 dilutions of bovine antiserum and a saline control were injected into each guinea pig. After 4 hours antigen or a mixture of antigens in 0.5% of Evans Blue solution (1 ml total volume) was injected into the foot vein. Reactions were read 15 to 30 minutes later either in the living animal or after sacrificing the animal and removing the skin. The degree of the reaction was graded according to size and intensity of discoloration as follows:

Area 15 to 20 mm in diameter, dark blue, 4+; area 10-15 mm in diameter, light blue, 3+; area 5-10 mm in diameter, light blue, 2+; area 2-5 mm in diameter, very light blue, 1+; no reaction, 0.

The drugs used to inhibit the PCA reaction were usually given 30 minutes prior to the challenge with the antigen. At least 5 guinea pigs were used in each test group. Scores expressing the degree of the reaction observed in individual guinea pigs of the group were added. This figure was called the total score. The percent suppression was derived by expressing the total score observed in the test animals as a percentage of the total score of the control animals.

The antisera utilized were prepared by immunizing New Zealand white rabbits weighing 2 to 3 kg with a suspension of penicillin G in Freund's adjuvant according to the technique of Josephson(5) and of Levine and Ovary(11). For use as antigen, potassium penicillin G (KPG) was conjugated with human gamma globulin (HGG) or bovine serum albumin (BSA) according to the technique of Levine(8); for this purpose KPG was incubated with the appropriate protein at pH 11 for 2 hours at room temperature, followed by dialysis in 0.02 M K_2HPO_4 buffer (pH 8.2) at 5°C.

The titer of the antisera was measured in two ways: by the passive hemagglutination, as described by Levine *et al*(10), and by the PCA response. The PCA titer recorded was the highest dilution of antiserum which produced a 3+ skin reaction in 50% of the guinea pigs.

Results. Effect of chlorphenesin on penicillin hypersensitivity. A typical experiment illustrating the action of 3-*p*-chlorophenoxy-1, 2-propanediol (chlorphenesin) is shown in Table I. A control group of 5 guinea pigs received intracutaneously dilutions of a penicillin antiserum and at other sites dilutions of a BSA antiserum. Another group of 5 guinea pigs received chlorphenesin 100 mg/kg intraperitoneally 3.5 hours after the intracutaneous injections of the antisera. Thirty

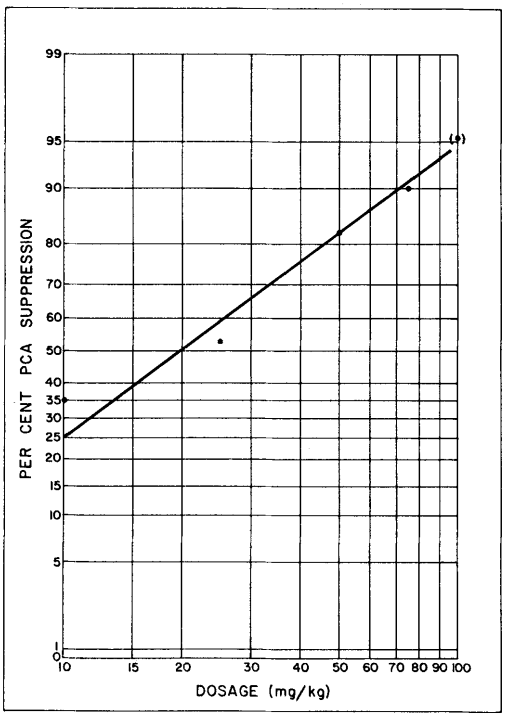

FIG. 1. Suppression of penicillin induced PCA response by graded doses of chlorphenesin. Guinea pigs. Chlorphenesin given intraperitoneally 30 min prior to antigen.

minutes later (or 4 hours after sensitization) both groups of animals were challenged intravenously with 1 ml of a mixture containing KPG-HGG conjugate 10 mg, BSA 1 mg and Evans blue 5 mg. The reaction read 30 minutes later showed in all control animals distinct blue patches at the sites injected with KPG or BSA antisera. In the group treated with chlorphenesin, reactions indicative of penicillin hypersensitivity were completely prevented; however, reactions to bovine serum albumin were not affected.

Fig. 1 illustrates the dependence of the suppression of the PCA reaction on the amount of chlorphenesin administered. In this series of tests, complete suppression of the PCA reaction was achieved after administration of chlorphenesin in doses of 100 mg/kg intraperitoneally. Chlorphenesin also affected the PCA reaction when administered by the intravenous or oral routes.

The interval of time between administration of the drug and injection of the antigen also affected the suppressant effect of chlorphenesin. Suppression of the PCA reaction was most pronounced when chlorphenesin was given 15 to 30 minutes before challenge. However, significant protection was also obtained when the drug was given at the time of challenge with penicillin.

The suppressant effect of chlorphenesin was related to the amount of antibody present in the antiserum. The higher the antibody titer, the more chlorphenesin was needed for complete suppression of the reaction. The PCA titer of antisera, however, did not necessarily correlate with the hemagglutination titer.

Chlorphenesin was not equally effective in inhibiting PCA reactions with all of the 12 penicillin rabbit antisera that have been prepared and titrated. The effectiveness of chlorphenesin 100 mg/kg intraperitoneally given 30 minutes prior to challenge with antigen, was evaluated with each antiserum in at least 3 separate trials. Six or more titrations were carried out with each serum. With 2 of the 12 antisera, suppression of the PCA reaction by chlorphenesin was very marked and varied between 90 and 100%. Two other antisera produced an average suppression of 87%, and 2 additional antisera 78%. The remaining 6 antisera produced, on the average, a suppression of only 67% or less.

The ability of chlorphenesin to suppress the penicillin-induced PCA reaction was also evaluated with a few antisera produced without the use of Freund's adjuvant. Several antisera obtained by immunization with conjugates of KPG with human gamma globulin, bovine gamma globulin or methylated bovine serum albumin, produced PCA reactions in guinea pigs that could not be easily inhibited by chlorphenesin.

Effect of chlorphenesin on PCA reactions induced by sera from penicillin hypersensitive humans. Through the courtesy of Dr. B. B. Levine, we obtained samples of sera (E-105A and G-38A) from 2 patients exhibiting penicillin hypersensitivity. These sera, injected intracutaneously to guinea pigs, produced definite PCA reactions on challenge with KPG-BSA conjugate. Administration of chlorphenesin 100 mg/kg intraperitoneally 30

TABLE II. Effect of Chlorphenesin on PCA Reactions Induced by Antisera from Penicillin Hypersensitive Humans.

	Antisera dilution								
	Control-untreated			Chlorphenesin treated					
Antisera	1/25	1/50	1/100	1/25	% Supp	1/50	% Supp	1/100	% Supp
E-105A	16*	13	9	7	56%	4	69%	2	78%
G-38A	12	10	9	8	33%	4	60%	2	78%

* Five guinea pigs were used in each test group.

minutes prior to challenge produced significant suppression of the PCA reaction (Table II).

Effect of related compounds. A number of compounds chemically closely related to chlorphenesin also decreased the penicillin-induced PCA reaction. We have evaluated a number of substituted 1-phenoxy-2-propanols, 3-phenoxy-1-propanols and 3-phenoxy-1,2-propanediols. The action of these compounds appeared to be similar. Because the degree of inhibition of the penicillin hypersensitivity reaction elicited by chlorphenesin was studied most thoroughly, the work reported in this paper was limited to it.

The mode of action of chlorphenesin. Chlorphenesin and other phenoxy propanediols could interfere with the PCA reaction in a number of ways. They may react with penicillin to destroy or inactivate it; they may combine with the antigen or antibody by blocking the reactive sites, or they may prevent the reaction between antigen and antibody. They may mask the detection of the reaction by neutralizing the end products of the antigen-antibody reaction or they may act by depressing the general reactivity of the animal or by exercising an anti-inflammatory action.

Interactions with penicillin. Possible interaction between penicillin and chlorphenesin in solution was investigated by an examination of the ultraviolet and infrared absorption of these 2 agents individually and in combination.

Ultraviolet spectra of an aqueous solution containing KPG 6.6 mg/ml and of a solution containing chlorphenesin 20 μg/ml were obtained. Optical addition of the 2 compounds in solution was effected by placing the 2 sample cells in the ultraviolet beam simultaneously. Readings were also obtained after the 2 solutions were combined and repetitive spectral scans were made for a period of 2 hours at 37°C. Stoichiometric ratios of the 2 substances were also examined in this manner. No evidence suggesting the formation of a complex was found.

Infrared spectra of solid residues of KPG and chlorphenesin individually and in combination were obtained by scanning KBr coated with the solid residues by lyophilization. No evidence of complex formation was apparent from these spectra.

The effect of chlorphenesin on the antibiotic action of penicillin was studied *in vitro* and *in vivo*. In the *in vitro* studies dilutions of penicillin ranging from 1 unit per ml up to 100,000 units per ml were incubated for 90 minutes with chlorphenesin solution containing 5 mg per ml. Utilizing the standard filter paper disc penicillin assay on agar plates seeded with *Staphylococcus aureus* or *Streptococcus mastitidis*, no alteration of the antibiotic activity of penicillin was noted. Thus, chlorphenesin, under conditions of the experiment, did not destroy or affect the antibiotic activity of penicillin. Chlorphenesin 5 mg/ml alone did not inhibit bacterial growth under these conditions.

The effect of chlorphenesin given jointly with penicillin was studied in mice infected with *Streptococcus mastitidis*. When penicillin, 10 mg/kg intraperitoneally, was given 4 hours after infection and then twice daily for 3 days, 90% of the infected animals survived during a 6-day period of observation. Such survival was not significantly affected when penicillin was administered jointly with chlorphenesin 100 mg/kg to another group of 20 mice (survival 95%) (Table III). Similar results were obtained with mice infected with *Klebsiella pneumoniae*.

TABLE III. Effect of Chlorphenesin on Antibiotic Action of Penicillin G in Mice Infected with *Streptococcus mastitidis* (520 Viable Cells = 50 × LD_{50}).

mg/kg penicillin G	mg/kg chlorphenesin	No. dead/No. tested Day 1	Day 3	Day 6	Survival, %
—	—	13/20	19/20	19/20	5
10	—	0/20	0/20	2/20	90
—	100	17/20	19/20	19/20	5
10	100	0/20	0/20	1/20	95

Interactions with antibody. Chlorphenesin might interfere with the penicillin-induced PCA reaction by blocking reactive sites on the antibody molecule. This hypothesis was tested *in vitro* and *in vivo*. In the *in vitro* studies penicillin antisera were first absorbed with 4 volumes of 6% washed human red blood cells for 15 minutes at 22°C to eliminate any naturally occurring agglutinins. Serial dilutions of the antisera with chlorphenesin were then prepared to give a final concentration of chlorphenesin 0.1% w/v. After incubation of the antiserum-chlorphenesin mixture for one hour at 37°C, penicillin-coated human red corpuscles were added, the mixture held at 37°C for one hour and stored overnight at 5°C. In these tests, illustrated in Table IV, incubation of penicillin antisera with chlorphenesin markedly reduced their ability to agglutinate penicillin-coated red cells. Antiserum #90 that had a hemagglutination titer of 1 in 1600, after incubation with chlorphenesin showed a reduction of the titer to 1 in 200. Such a reduction was specific in this and 5 replicate tests as incubation of BSA antisera with chlorphenesin did not cause a reduction of specific hemagglutination titers.

The effect of incubation of chlorphenesin with penicillin antisera prior to their utilization in the PCA reaction was also evaluated. Serial dilution of a penicillin antiserum (#90) was prepared with saline containing the proper amount of chlorphenesin to give a final concentration of 0.1% w/v of the drug. The mixtures were incubated for one hour at 37°C and injected intradermally to guinea pigs for PCA testing. The animals were challenged after one hour with a KPG-BSA conjugate. The antiserum, preincubated with chlorphenesin, produced a weaker PCA reaction than a similarly treated portion of the antiserum that had not been incubated with the compound (Table V). When, however, the animals were challenged 4 hours after sensitization, as is usually done, no suppression of the PCA reaction with sera preincubated with chlorphenesin was noted (Table V). The differences observed between animals that were challenged at 1 or at 4 hours after sensitization appear to indicate that the antibody-chlorphenesin interaction is of a labile nature. The reaction was, however, specific to penicillin as PCA reactions carried out with anti-BSA sera preincubated with chlorphenesin were not affected. Immuno-suppressive agents, such as methotrexate, cyclophosphamide, 6-mercaptopurine or 6-thioguanine, did not affect the penicillin-induced PCA reaction in guinea pigs (Table VI).

Other mechanisms. It is well known that the union of antigen with antibody at certain sites is often accompanied by a release of biogenic amines, such as histamine, acetylcholine, serotonin and others. It has previously been shown that the PCA reaction in the guinea pig can be inhibited by administration of antihistaminics(13). Chlorphenesin 20 μg/ml, however, when evaluated on the isolated guinea pig ileum, was unable to antagonize the spasmogenic effect of histamine 0.01 μg/ml, acetylcholine 0.02 μg/ml or serotonin 2.0 μg/ml. Under these conditions, diphenhydramine HCl 0.05 μg/ml, atropine sulfate 0.004 μg/ml and chlorpromazine HCl 1.0 μg/ml antagonized spasms pro-

TABLE IV. Effect of Preincubating Penicillin G Antiserum #90 with Chlorphenesin on Hemagglutination Titers.

Treatment	Hemagglutination titer at antisera dilution of 1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Control (saline)	4+	3+	3+	2+	1+	0	0
Chlorphenesin (0.1%)	4+	1+	0	0	0	0	0

TABLE V. Effect of Incubating Penicillin Antiserum #90 with Chlorphenesin Prior to PCA Testing.

Time of challenge after sensitization "hours"	Antiserum dilutions: Control antisera 1/100	Control antisera 1/300	Chlorphenesin pretreated antisera 1/100	% Suppression	Chlorphenesin pretreated antisera 1/300	% Suppression
1	15*	9	11	27	5	44
4	19	14	19	0	19	0

* Five guinea pigs were used in each test group. The figures represent sums of individual PCA scores.

TABLE VI. Effect of Immuno-Suppressive Compounds on Penicillin G-Induced PCA Reactions in Guinea Pigs.

Treatment*	Antiserum dilutions: 1/100 Score	1/100 % Supp	1/300 Score	1/300 % Supp	1/900 Score	1/900 % Supp
Saline control	17	0	12	0	6	0
6-Thioguanine (3 mg/kg)	19	0	13	0	6	0
6-Mercaptopurine (50 mg/kg)	20	0	14	0	10	0
Methotrexate (10 mg/kg)	17	0	14	0	8	0
Cyclophosphamide (100 mg/kg)	18	0	13	0	4	33
Chlorphenesin (100 mg/kg)	3	82	1	92	0	100

* All treatment administered by intraperitoneal route 30 min prior to antigen challenge.

duced by histamine, acetylcholine and serotonin, respectively. Chlorphenesin 300 mg/kg intraperitoneally was ineffective in preventing bronchospasm and death induced in guinea pigs by intravenous injection of histamine 1.1 mg/kg given 30 minutes after the drug. The antihistaminic chlorpheniramine maleate 0.3 mg/kg intraperitoneally afforded complete protection under these circumstances.

Chlorphenesin, like mephenesin(2) to which it is very closely related, when given to mice, rats or guinea pigs intraperitoneally in doses of 200 mg/kg or more, produces muscular relaxation and paralysis of voluntary muscles by a depressant effect on the interneurons of the central nervous system. However, the suppression of penicillin hypersensitivity by chlorphenesin has been observed after doses of 100 mg/kg that do not elicit muscular relaxation or paralysis. The effect of the drug cannot be ascribed to a generalized depressant effect because other central nervous system depressants, such as pentobarbital, do not suppress the penicillin hypersensitivity reaction in guinea pigs even when given in doses of 20 mg/kg which produce hypnosis and anesthesia.

The PCA reaction has the characteristics of an acute inflammatory reaction. Thus, chlorphenesin might suppress the PCA reaction by exercising an anti-inflammatory action. Chlorphenesin 120 mg/kg intraperitoneally given to 12 rats 18 hours after injection of silver nitrate into the ankle joint according to the method of Margolin(12) did not exert any anti-inflammatory action. In the kaolin foot pad test(4), chlorphenesin 100 mg/kg given intraperitoneally to 6 rats one hour before and one hour after the kaolin, did not decrease the volume of edema as measured 5 hours after the kaolin injection. In addition, anti-inflammatory agents such as aspirin 100 mg/kg, aminopyrine 100 mg/kg intraperitoneally given 30 minutes prior to challenge and cortisone 25 mg/kg given subcutaneously 18 and 3 hours prior to challenge did not suppress the appearance of the penicillin-induced PCA reactions in groups of 10 guinea pigs. It may be concluded that chlorphenesin does not have an anti-inflammatory action and does not suppress penicillin hypersensitivity reactions by virtue of an anti-inflammatory effect.

Discussion. At the present time penicillin causes more hypersensitivity reactions than

any other drug. It has replaced foreign sera as the most common cause of anaphylactic shock(14). The understanding of the nature of these reactions and their prevention is thus of great practical importance.

The passive cutaneous anaphylaxis reaction appears particularly suitable for the study of penicillin hypersensitivity. In this test, as shown by Josephson *et al*(6) only the 7S antibodies—which are responsible for the symptoms of hypersensitivity and anaphylaxis—can sensitize the guinea pigs. The 19S antibodies that can produce hemagglutination but are not responsible for symptoms of hypersensitivity in humans, cannot fix to the skin of guinea pigs and do not play a part in the PCA test. Results obtained in the PCA reaction in the guinea pig thus should correlate with the anaphylactic reactions and particularly with the dangerous immediate systemic reactions observed in humans(9).

There is no test available that would reliably detect individuals who will react with an anaphylactic reaction to subsequent administration of penicillin(3,8). Under these circumstances, a non-toxic substance administered concomitantly with penicillin, that could prevent symptoms of anaphylaxis, would be of great value. It would also be necessary that such a substance would not interfere with the antibiotic activity of penicillin, would not elicit untoward symptoms in subjects who are not hypersensitive to penicillin and would not affect other immunological mechanisms. The results reported here indicate that chlorphenesin may fulfill these requirements. However, a full evaluation of the preventive and therapeutic potentials of this substance will require clinical trials in human beings.

Even more important than the possible clinical usefulness is the mode of action of this compound. Although the details have not been fully elucidated, it would appear that chlorphenesin inhibits hypersensitivity reactions in a manner dissimilar to that of any other known agent that is not a hapten and is unrelated to the antigen itself. The drug does not inactivate penicillin and does not act by neutralizing or making the body insensitive to the end products of antigen-antibody reactions such as histamine, acetylcholine or serotonin. It does not suppress penicillin hypersensitivity by decreasing the general reactivity of the organism. Chlorphenesin apparently acts by blocking or otherwise affecting certain receptor sites of penicillin antibodies. The antibody or antibodies that are affected belong to the 7S class and play an important role in penicillin hypersensitivity. Chlorphenesin has little affinity for antibodies against BSA or *S. typhosa* as it does not affect PCA reactions elicited by them and their specific antigens. The union between chlorphenesin and the penicillin antibody must, however, be a loose one because of the ease with which this complex apparently dissociates in the animal body. This is indicated by the finding that the penicillin antibody-chlorphenesin complex injected intracutaneously showed partial suppression of the PCA reaction when challenged after 1 hour but no suppression on challenge 4 hours after sensitization.

The present paper indicates that it is possible to suppress selectively antigen-antibody reactions *in vivo* by the systemic administration of simple chemical compounds. A further study of the exact mechanism of the mode of action of these substances may not only help to further our understanding of the structure of antibodies and of the specificity of immunological reactions, but may also lead to the development of drugs useful in the control of hypersensitivity, allergy and auto-immune diseases.

Summary. Passive cutaneous anaphylaxis (PCA) elicited in guinea pigs with penicillin conjugates can be selectively suppressed with certain simple phenoxy propanediols. Of these, 3-*p*-chlorophenoxy-1,2-propanediol (chlorphenesin) was most extensively studied. Chlorphenesin elicited little interference with PCA induced by bovine serum albumin or *S. typhosa*. Many, but not all, of the rabbit antisera employed in these studies were subject to the interference by chlorphenesin. PCA reactions induced by 2 sera from penicillin hypersensitive humans were inhibited by chlorphenesin. The mode of action of chlorphenesin appears to be different from that of

previously described inhibitors of anaphylactic reaction. The compound does not destroy penicillin, does not affect its antibiotic action, is devoid of antihistaminic and anti-inflammatory activity, and does not act by a generalized depression of the reactivity of the organism. Incubation of penicillin antibody with chlorphenesin decreases the antibody titer; thus, chlorphenesin may act by blocking certain antibody sites and in this manner interfere with specific antigen-antibody reactions.

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Effects of Epinephrine and Acetylcholine on Hypothalamic Content Of Prolactin Inhibiting Factor.*† (31729)

JAMES C. MITTLER AND JOSEPH MEITES
Department of Physiology, Michigan State University, East Lansing

Virgin female rats injected subcutaneously for 10 days with 10 μg estradiol daily, followed by twice daily injections of 25 mg acetylcholine iodide/kg body weight or 0.25 mg epinephrine in oil for 5 days, showed extensive lobulo-alveolar mammary growth and lactation(1,2). Similar results were obtained when epinephrine, acetycholine iodide or serotonin were injected into estrogen-primed female rabbits(3). Epinephrine, acetylcholine and serotonin can also induce pseudopregnancy in rats under certain experimental conditions(4). The results are interpreted as indicating that these neurohumors stimulate release of prolactin from the pituitary.

The presence of a prolactin-inhibiting factor (PIF) in acid extracts of rat hypothalami has been demonstrated(5). The *in vitro* assay method developed for PIF indicated that the suckling stimulus during lactation(6) or injections of estrogen(6) or reserpine(7) significantly decreased the PIF content of the hypothalamus. Since it has been found that epinephrine and acetylcholine have no direct effect on the pituitary *in vitro* (5), it was of interest to determine whether their lactational effects were exerted by depressing the PIF content of the hypothalamus.

Materials and methods. The work reported here follows in detail the methods described previously(5,6) except where otherwise noted. For each of 2 experiments designed to investigate the effects of epinephrine, experimental hypothalamic donors were 12 Carworth CFN female rats injected subcutaneously with 0.25 mg epinephrine in oil (Parke-Davis Co.) twice daily for 5 days and killed

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