Suppression of the Phenomenon of Local Tissue Reactivity by ACTH, Cortisone and Sodium Salicylate. (18136)

GREGORY SHWARTZMAN, S. STANLEY SCHNEIERSON, AND LOUIS J. SOFFER.

From the Division of Bacteriology, Laboratories of The Mount Sinai Hospital, New York City.

It was recently reported that ACTH is capable of suppressing the phenomenon of local tissue reactivity, when administered intramuscularly 2 hours prior to the provocative injection of meningococcus culture filtrate(1). The experiments embodied in the following paper serve to extend these observations.

Experimental. Substances failing to inhibit or modify the phenomenon of local tissue reactivity to bacterial filtrates. The interpretation of the results of the present studies is facilitated by considering the failure of a variety of substances to inhibit or modify the phenomenon of local tissue reactivity.

In published(2) and unpublished experiments by one of the authors (G.S.), the substances employed were administered repeatedly by various routes. The substances which failed to suppress the phenomenon were acetylcholine, adenosine-5-phosphoric acid, alypin, antiplatelet serum, amino acids in various combinations, ascorbic acid, atropine, benadryl, biotin, calcium chloride, calcium gluconate, casein hydrolysate, choline, cocaine, congo red, curare, DCA, dicumarol, distilled water, estradiol, ether general anesthesia, folic acid, glucose, heparin, hesperidin, histaminase, histamine, india ink, inositol, lemon "citrin," milk, Niagara sky blue, nicotinic acid, pantothenic acid, paraminobenzoic acid, physostigmine hydrochloride, pilocarpine, progesterone, pyribenzamine, pyridin (in doses reducing significantly the blood platelet count), pyridoxal, pyridoxamine, pyridoxine, rat liver extract, rat spleen extract, riboflavin, sodium oxalate, sulfadiazine, sulfanilamide, sulfathiazole, thiamine, α-tocopherol, trypan blue, trypsin, urethane, vitamin K (water soluble and oil soluble preparations), wheat germ oil, and yeast extract. Becker(3) showed that BAL, mapharsen. partial exsanguination and thyroidectomy failed to inhibit the phenomenon, while Smith and Humphrey observed no effect with anthisan(4).

Substances capable of suppressing the phenomenon of local tissue reactivity. The bacterial preparations used for the preparatory and provocative injections were meningococcus, 44B, "agar washings" filtrates(2). Rabbits were prepared by a single intradermal injection of 0.25 ml of the filtrate diluted 1 : 2 and injected intravenously 24 hours later with 125 to 150 multiples of the minimal provocative dose. The doses employed gave large strongly positive reactions in over 90% of the control animals.

As may be seen from Table I, cortisone gave consistent inhibition of the phenomenon, when administered intramuscularly 2 hours prior to the provocative injection of the bacterial filtrate. Longer (Group 7) and shorter intervals (not recorded in the table) between the administration of cortisone and the provocative injection of the bacterial filtrate were less effective. The dose of cortisone required for consistent suppression was approximately 6 times greater than that of ACTH.

Lewin and Wassen(5) reported beneficial clinical effects similar to ACTH and cortisone from DCA and ascorbic acid. Thus we studied the effect of these substances on the phenomenon, (Group 1). The treatment failed to modify the phenomenon.

Smith and Humphrey(4) reported recently that sodium salicylate was capable of suppressing the phenomenon elicited with *E. coli*

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TABLE I. Suppression of the Phenomenon of Local Tissue Reactivity.

Group No.	Treatment			Results		
	Substance	Total dose	Method of administration	Positive	Doubtful	Negative
1	DCA and Sodium ascorbate	10 mg 1 g	I.M. 2 hr prior prov. inj. I.V. 10 min. prior prov. inj.	5	1	0
2	Cortisone	12.5 mg	I.M. 2 hr prior prov. inj.	4	2	0
3	"	25 "	"	2	1	3
4	"	40 "	"	4	2	0
5	"	50 "	"	2	0	1
6	"	75 "	"	1	1	4
7	"	60 "	I.M. 4 hr prior prov. inj.	2	0	1
8	Sodium salicylate	1.6 g/kg	I.P. 20 min. prior prep. inj. 18 hr, ½ hr prior prov. inj. and 2 hr after prov. inj.	12	0	17
9	Calcium pantothenate	0.8 "	"	6	0	0
10	Sodium salicylate and Calcium pantothenate	1.6 " 0.8 "	"	0	1	5
11	Sodium salicylate and Calcium pantothenate	1.6 " 0.4 "	"	0	0	10
12	Sodium salicylate and Calcium pantothenate	1.6 " 0.2 "	"	2	0	4
13	Sodium salicylate and Cortisone	1.6 " 50 mg	" I.M. 2 hr prior prov. inj.	1	0	5
14	ACTH	12.5 mg	I.M. 2 hr prior prov. inj.	2	0	14
15	Sodium salicylate and ACTH	1.6 g/kg 7.5 mg	I.P. 20 min. prior prep. inj. 18 hr, ½ hr prior prov. inj. and 2 hr after prov. inj. I.M. 2 hr prior prov. inj.	2	0	3
Controls for groups 1-15	—	—	—	65	3	3

Prep. inj. = Intradermal preparatory injection of the bacterial filtrate; prov. inj. =intravenous provocative injection of the bacterial filtrate; I.M. = intramuscularly; I.V. = intravenously; I.P. = intraperitoneally.

toxin. In some rabbits the reaction was completely inhibited, while in others the size of the reaction was markedly reduced. In our studies (Table I, Group 8) using a stronger toxin (meningococcus) the phenomenon was completely inhibited in 17 out of 29 rabbits, while in the remaining animals the reactions were strongly positive. The size of the reactions was not reduced.

Calcium pantothenate alone failed to produce any effect upon the phenomenon (Table I, Group 9). However, optimum combined doses of sodium salicylate and calcium pantothenate resulted in suppression of the phenomenon in all animals tested (Group II). Apparently, calcium pantothenate enhanced the suppressing effect of sodium salicylate without directly affecting the phenomenon.

Apparently the addition of sodium salicylate may enhance the effect of an insufficient dose of cortisone, while the action of ACTH in subminimal concentration is not improved by the addition of sodium salicylate (Groups 5, 13 and 15).

Discussion. The most important feature of the phenomenon of local tissue reactivity is profound vascular damage in the prepared site, which can only be elicited when the provocative injection is made into the general circulation. No satisfactory explanation is yet available concerning the necessity of introducing the provocative agent into the vascular system. Several possible mechanisms may be considered.

The possibility that a disturbance in the coagulation system is necessary for the elicitation of the reaction is not supported by the fact that agents capable of altering blood

coagulation, namely, distilled water, heparin, dicumarol, vitamin K, calcium salts, sodium oxalate, pyridine, and anti-platelet serum fail to produce any effect on the phenomenon. The role of the peripheral and central nervous system in the provocation of the phenomenon is excluded by lack of effect of general anesthesia and a variety of local anesthetics, atropine, acetylcholine, physostigmine, curare and pilocarpine. The suggestion that the reaction may be caused by release of histamine following the provocative injection is not borne out by the inability of histamine to provoke the reaction and the failure of histaminase and antihistaminics to exert any influence. Assumption receiving most support is that the damage to vascular endothelium necessary for the production of the reaction can be elicited only when the provocative agent is brought in contact with the endothelium intravascularly. Becker recently showed that HN_2, benzol and x-ray radiation(3) are capable of suppressing the phenomenon. He postulated that these agents produce the suppression by rendering the vascular endothelium anergic in the prepared site, thus preventing it from reacting to the provocative agent. Recently Schlang(6) confirmed the observations of Becker. In studying the mechanism of this inhibition he found that protection of the prepared site from the effect of HN_2 failed to influence the inhibition of the reaction. In contrast, protection of the lower limbs from the action of HN_2 by aortic occlusion decreased or prevented the inhibition. He concluded that the effect of HN_2 was the result of systemic rather than local action.

Our observed suppression of the phenomenon by ACTH and cortisone indicates that provocation of the phenomenon depends on adrenal cortical function. Then the reported suppression of the phenomenon by sodium salicylate needs further examination. Drugs and certain environmental factors are capable of influencing adrenal cortical function, as judged by the decrease in concentration of adrenal ascorbic acid(7). Possibly then, the suppressing effect of sodium salicylate on the phenomenon is indirect, being mediated by the adrenal cortex. Thus, we examined the effect of pantothenic acid. Pantothenic acid deficiency produces adrenal cortical damage(8) and probably some adrenal cortical disfunction. According to Gaunt, Liling and Mushett(9), rats on a diet deficient in pantothenic acid show a decreased resistance to water intoxication. Sodium salicylate gave suppression of the phenomenon only in 17 out of 29 rabbits tested. Since the effect of sodium salicylate may be mediated through the adrenal cortex it seemed of interest to determine the effect of pantothenic acid on the action of sodium salicylate. Pantothenic acid enhanced the suppressive action of sodium salicylate on the phenomenon, since pantothenic acid in itself fails to show any effect.

Although non-anaphylactic in nature, the phenomenon is due to a profound alteration in vascular reactivity. Direct and indirect clinical evidence strongly suggests that the phenomenon demonstrates the mechanism underlying the production of a variety of spontaneous diseases and syndromes of known and unknown etiology in which vascular lesions are a predominant feature(1,10). These diseases fall into the spectrum of therapeutic activity of ACTH and cortisone. The ability of the agents to suppress the phenomenon points again to the role the phenomenon plays in the elicitation of these diseases and at the same time offers an experimental approach for the elucidation of the beneficial therapeutic effect of these

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agents. Since the combined treatment with sodium salicylate and calcium pantothenate proved effective experimentally, the clinical trial of the combination may be worthy of consideration.

Summary. ACTH, cortisone and sodium salicylate were capable of suppressing the phenomenon of local tissue reactivity. The dose of cortisone required for the inhibition was approximately 6 times greater than that of ACTH. Sodium salicylate completely inhibited the phenomenon in 17 out of 29 rabbits while in the remaining animals the reactions were strongly positive. Pantothenic acid, which had no effect upon the phenomenon by itself, enhanced significantly the suppressing effect of sodium salicylate.

Technical assistance of Miss Joan Greenwald is thankfully acknowledged.

Received July 11, 1950. P.S.E.B.M., 1950, v75.

Demonstration of Increased Gonadotrophic Hormone Production in Castrated Mice with Intrasplenic Ovarian Grafts.* (18137)

O. J. MILLER AND CARROLL A. PFEIFFER.

From the Department of Anatomy, Yale University, New Haven.

Granulosa cell tumors, luteomas and mixed tumors occur in intrasplenic or intrapancreatic ovarian grafts in castrated rats(1) and mice(2). It has been demonstrated that the livers of animals of these species can inactivate estrogenic hormones(3-5) and that castrated rats or mice with intrasplenic ovarian grafts remain in diestrus after the first few weeks from the time of grafting(1,2). Therefore, it has been concluded that the pituitary glands of these animals are of the castrate type and produce greatly increased amounts of gonadotrophic hormones, with the resultant formation of ovarian tumors. In support of this concept, it has been shown (1,2,6) that the presence of an intact ovary, or of vascular adhesions between the graft and the body wall or the uterus, or the administration of estrogenic or androgenic hormones prevents the occurrence of ovarian tumors in intrasplenic ovarian grafts. No direct determination of the rate of production of gonadotrophic hormones in castrated animals with intrasplenic ovarian grafts has been reported. However, Jungck, Heller and Nelson(7) bioassayed the pituitary glands of castrated rats with intrasplenic ovarian grafts and found only a slight increase in gonadotrophic potency, with an equal increase in the rats with adhesions between the graft and the body wall. Their experiment was not critical, since the gonadotrophic content of the pituitary gland may not be proportional to the rate of release of gonadotrophins from it(8). The technic of parabiosis provides a method for assaying circulating gonadotrophins. The output of pituitary gonadotrophins is increased in castrated(9) and x-rayed(10) rats as de-

* This investigation was aided by grants from The Anna Fuller Fund, the National Cancer Institute (U.S.P.H.S.), and the James Hudson Brown Fund of Yale University School of Medicine.

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