STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS

III. Anaphylaxis Induced by Arsphenamine

By K. LANDSTEINER, M.D., and JOHN JACOBS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

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Reference has been made in a previous paper (1) to the work of several authors (Swift, Frei, Sulzberger, Mu) on sensitization to arsphenamine and neoarsphenamine. In most of the communications skin lesions or "flare ups" at the site of old injections were described. Of 19 animals given intraperitoneal or intravenous injections of neutralized arsphenamine mixed with guinea pig serum Swift (2) observed, on reinjection of a similar mixture, "symptoms like those seen in anaphylaxis;" one animal succumbed in 10 minutes, and two others after some hours. Arsphenamine alone, without serum, did not seem to sensitize. Kolle and Rothermund (3) after unsuccessful attempts obtained positive results by sensitizing with very small quantities of neosalvarsan and observed acute death with symptoms which had "a certain similarity to anaphylactic shock" (translated). These experiments, briefly mentioned in a discussion on allergy, have not, to our knowledge, been published in detail.

Lauf (4) injected neosalvarsan solutions without serum subcutaneously and upon intravenous reinjection observed severe anaphylactic symptoms in perhaps 15 per cent of the animals and in others slight symptoms, but in no instance did death occur in anaphylactic shock. The most striking results were obtained after a rest period of 6 months.

Working with the guinea pig uterus (Schulz-Dale method) Kallós and Kallós-Deffner (5) found that reactions occurred in 7 of 11 cases after previous subcutaneous treatment with horse serum-arsphenamine mixtures. For the tests, also, serum mixtures were used. Anaphylactic experiments other than those with the Schulz-Dale method are not mentioned. According to the authors, sensitization could be transferred passively.

Birnbaum (1934), who gives a comprehensive review of the literature (6), concludes that there is no proof for the assumption that skin manifestations in human beings following administration of arsphenamine are true allergic effects, and casts doubt on the reports concerning passive transfer of arsphenamine hypersensitiveness. With regard to animal experiments he remarks that "it has never

been possible, even with large doses of arsphenamine, . . . to produce anaphylactic shock" (translated).

On account of the irregularity of effects, as recorded in the literature, and the differences between results of various investigators, attempts directed toward improving the technique were thought to be desirable.

We found that, with a suitable method, intense local hypersensitive-

TABLE I*

Sensitization effects in two batches of guinea pigs similarly treated with one intracutaneous injection of 0.15 mg. arsphenamine, not neutralized, and neoarsphenamine, respectively, in 0.1 cc. saline; tested after an interval of 1 month with the respective substances.

No.	Animals sensitized to and reinjected with arsphenamine	No.	Controls
1	22, p.p.–p., el., necr.c. 4	7	6, p.p., el.
2	12, p., el., necr.c. 3	8	6, p.p., el.
3	9, p., el., necr.c. 3	9	7, f.p., sl.el.
4	26, p., swol., necr.c. 3	10	5, p.p., el.
5	18, p., el., necr.c. 3	11	5, f.p., el.
6	19, p.p., el., necr.c. 3	12	5, f.p., el.
No.	Animals sensitized to and reinjected with neoarsphenamine	No.	Controls
13	5, p.p., sl.el.	19	6, f.p., el.
14	5, f.p., sl.el.	20	6, a.cls., fl.
15	4, a.cls., sl.el.	21	5, f.p., el.
16	a.neg.	22	4, f.p., sl.el.
17	5, f.p., el.	23	6, p.p., sl.el.
18	6, p., el.	24	6, p.p., sl.el.

* The following abbreviations are used: almost colorless (a.cls.), faintly pink (f.p.), pale pink (p.p.), pink (p.). Other designations are: almost negative (a.neg.), flat (fl.), slightly elevated (sl.el.), elevated (el.), necrotic center (necr.c.). The figures give diameters of the lesions in millimeters.

ness could regularly be obtained (1). With arsphenamine solutions, not neutralized, practically all sensitized animals gave conspicuous lesions consisting of rather large pink elevated areas almost regularly with central necrosis. When compared with effects produced by neoarsphenamine, it appeared that arsphenamine had a distinctly greater sensitizing capacity. This is evidenced by the experiment given in Table I.

Strongly positive skin reactions to arsphenamine were observed in seven experiments with more than 50 animals which in part are shown in Table II. On the first injection only faint to pale pink lesions were produced, with a diameter of 4–7 mm.

These observations prompted attempts to produce anaphylaxis in animals sensitized by this method. Preliminary experiments indicated that several injections extending over a rather long period were effective; the technique developed was as follows.

Guinea pigs were given 2 intracutaneous injections of 0.15 mg. arsphenamine, not neutralized, in 0.1 cc. saline a month apart and 3 weeks later injected intravenously with a solution of 10 mg. arsphenamine in 0.1 cc. saline and 0.09 cc. N/1 NaOH, made up to 1.0 cc. with normal guinea pig serum. In those animals which did not succumb to the first intravenous injection the dose was repeated after 3 weeks.

The results obtained with three batches of guinea pigs treated with the same brand of arsphenamine and by the same method, are given in Table II.

About half of the animals died in typical anaphylaxis on either the first or second injection, while still others presented distinct symptoms. It may be of interest to note that the number of guinea pigs with no or very slight symptoms, or those which died in shock, was greater than the number of animals showing symptoms of medium degree.

Of 50 controls injected intravenously in the same way as the animals previously treated, 35 showed no symptoms at all, 13 gave coughs, and 2 were slightly sick. The following drops in temperature of 0.5°C. or more were shown by one animal each: $-0.5°$, $-0.6°$, $-0.7°$, $-1.2°$, and $-1.4°$.

When, in another batch of sensitized animals, instead of a guinea pig serum-arsphenamine mixture 5 mg. of arsphenamine, not neutralized, was injected intravenously again some of the animals succumbed with anaphylactic symptoms, seeming to indicate that serum admixture is not necessary for the success of the experiment (Table III). Whether it offers a distinct advantage remains to be investigated in more extensive experiments.

In all, of 56 treated guinea pigs, 30 died in typical anaphylaxis, and a number of others showed distinct symptoms, whereas, as already

indicated, none of the 50 controls injected in the same manner as the treated guinea pigs died or developed severe symptoms.

TABLE II

Combined table. Sensitization with arsphenamine (Winthrop Chemical Company). The second column gives the diameter of the skin lesions in millimeters. Figures in parentheses indicate change in temperature (°C.).

No.	Second intracutaneous injection	First intravenous injection	Second intravenous injection
		Symptoms	Symptoms
	mm.		
25	22	None (−0.1)	None (0)
26	22	" (−0.9)	" (−0.5)
27	14	Slight (−1.9)	† 7 min.
28	17	" (−1.0)	† 8 "
29	23	† 19 min.	
30	18	Slight (−1.1)	Slight (−0.5)
31	16	None (−0.4)	† 8 min.
32	7	" (0)	Moderate (+0.2)
33	14	Slight (−2.7)	Severe (−3.7)
34	21	" (−1.8)	None
35	20	† 6 min.	
36	22	† 3 "	
37	10	Moderate (−1.6)	Slight (0)
38	25	† 20 min.	
39	21	Moderate (−1.0)	None (+0.2)
40	15	† 5 min.	
41	25	† 7 "	
42	9	† 4 "	
43	17	Slight (0)	† Overnight
44	27	† 38 min.	
45	7	None (+ 0.1)	None (0)
46	18	" (0)	" (0)
47	17	† 19 min.	
48	21	None (0)	None (+0.2)
49	20	† 4 min.	

† Designates death. Animals dying in a short time all showed the typical picture (lungs inflated, heart beating) on autopsy.

In a similar experiment with 5 animals an arsphenamine preparation, procured from the Abbott Laboratories, was used. In this lot 2 animals died on the second intravenous injection.

A point of special significance is that in the experiments reported a synthetic chemical substance, without being used in chemical combination with protein, produces skin sensitization as well as the anaphy-

lactic state. It will be of interest to follow up the mechanism of this sensitization.

TABLE III

Sensitization effects in a batch of guinea pigs treated with two skin injections a month apart of 0.15 mg. arsphenamine, not neutralized, (Winthrop Chemical Company) and 3 weeks after the second injection, injected intravenously with 5.0 mg. of the same preparation in 1.0 cc. saline. The second, similar intravenous injection was given 3 weeks after the first. Figures in parentheses indicate change in temperature (°C.).

No.	First intravenous injection	No.	Second intravenous injection
	Symptoms		Symptoms
50	Slight (−3.5)		None (0)
51	None (0)		Moderate (−1.0)
52	" (−0.1)		Coughs (0)
53	† 8 min.		
54	Slight (−2.5)		† 16 min.
55	Moderate (−4.4)		† 51 "
56	None (0)		Coughs (+0.1)
	Controls		Controls
57	None (−0.1)	64	None (0)
58	" (+0.1)	65	" (+0.2)
59	" (+0.2)	66	" (+0.1)
60	" (+0.1)	67	" (0)
61	" (−0.1)	68	" (0)
62	" (0)	69	" (+0.1)
63	" (0)	70	" (0)

SUMMARY

Experiments are described which show that with a given treatment guinea pigs can be sensitized to arsphenamine, so that a considerable percentage die in anaphylactic shock on intravenous administration of the substance.

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