other. All the animals but 2 were made unconscious one or more times during the course of the experiments and died shortly after as an immediate consequence of the trauma. In one case, (rat 6), the brain damage consisted of extensive contused lacerations, in another (rat 8) a subarachnoidal hemorrhage was found, while in a third (rat 46) multiple petechiae of the ring type were seen irregularly scattered both in the cortex and in the subjacent white matter of the cerebral hemispheres. Various stages of a fundamentally identical neurolytic change affecting, in a disseminated fashion, both nerve cells and nerve fibers, were displayed in almost all of the remaining animals, by present histological methods. If it is true that vagal, sympathetic and parasympathetic stimuli of central origin, (Cushing[1]), are responsible for the development of the gastric changes together with more striking disturbances in the circulatory system, the collateral findings of acute pulmonary edema and of petechiae in the serous membranes in 6 out of the 14 animals are significant, emphasizng the role of the neurocirculatory disturbances elicited by mechanical brain injury. Of these 6 animals, one died 5 minutes after the trauma, another after 8 minutes. These were the earliest casualties showing evidence of gastric damage. Most of the animals displaying a similar finding died between the 1st and the 7th day, and no hemorrhages or erosions were found in the rats dying or killed beyond the 29th day (the majority of the animals). These observations, in agreement with the findings of others, support the opinion most generally accepted (Moritz[6]) that if recovery from the head injury occurs and the acute effects of the gastric changes are survived, the tendency of the latter is toward prompt and complete healing.

Summary. Hemorrhagic changes, less often erosions, in the gastric mucosa were found in a number of rats submitted to blunt impacts on the head, single or repeated, of different intensities and according to different mechanisms. A certain correlation was seen between the severity of the trauma, correspondingly of the cerebral damage, and the occurrence of the gastric changes, which, when the acute effects of the trauma were survived, showed a tendency towards prompt and complete healing.

6 Moritz, A. R., *The Pathology of Trauma*, Lea & Febiger, Phila., 1942.

14780

Experiments on the Sensitizing Properties of Penicillin.

WILLIAM T. MCCLOSKY AND M. I. SMITH.

From the Division of Physiology, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

In the clinical application of penicillin toxic reactions are not very common. Nevertheless, reactions have been observed which are at least suggestive of an allergic manifestation. Keefer and associates[1] stated that in the treatment of 500 cases with penicillin toxic effects were rare, though occasional chills with fever, headache, and flushing of the face, muscle pain and constriction of the chest, and urticaria have been noted. Cohn[2] reported no toxic effects in a series of 48 cases, while Dawson and Hobby[3] observed occasional instances of various reactions in a series of 100 cases. Usually the toxic reactions were of a mild nature. Urticaria was recorded in 3 cases. In discussing toxic reactions from penicillin, Lyons[4] noted urticaria with fever

1 Keefer, C. S., Blake, F. G., Marshall, E. K., Jr., Lockwood, J. S., and Wood, W. B., *J. A. M. A.*, 1943, **122**, 1217.

2 Cohn, A., Studdiford, W. E., and Greenstein, I., *J. A. M. A.*, 1944, **124**, 1124.

3 Dawson, M. H., and Hobby, G. L., *J. A. M. A.*, 1944, **124**, 611.

TABLE I.

Anaphylactic Response to Intravenous or Intracardiac Reinjection of Penicillin. All Animals Also Sensitized to Horse Serum.

No.	Sensitizing dose, units	Incubation period, days	Shocking dose, units	Result	Shocking dose of horse serum, cc	Result
1	1 x 300 + 1 x 400 + 1 x 500 i.p.	30	5000 i.v.	No symptoms	0.5	Died in 3 min.
2	1 x 300 + 1 x 400 + 3 x 500 "	30	8350 i.c.	Died in 5 min.		
3	"	31	21400 "	Symptoms with recovery	0.5	" " 7 min.
4	" s.c.	30	9250 "	Immediate symptoms. Death in 72 min.*		
5	" "	30	12050 "	No symptoms	0.04	" " 5 min.
6	" "	30	23640 "	Severe typical symptoms with recovery	0.5	Severe symptoms with recovery
7	" "	30	9800 "	No symptoms	0.04	Severe symptoms with recovery
8	" "	30	11450 i.v.	" "	0.1	No symptoms
9	" i.p.	30	8850 i.c.	Mild "	0.04	" "
10	" "	30	9100 "	No "	0.02	" "
11	" "	30	8100 "	Mild "	0.04	Moderate symptoms

* Intrapleural hemorrhage at necropsy.

and other symptoms in 5.7% of 209 cases which he considers an atypical sensitization phenomenon because of the transient character of the period of sensitivity.

The unexplained nature of the toxic reac-

FIG. 1.

Guinea pig sensitized by five intraperitoneal injections of 500 Oxford units each of penicillin given at daily intervals. Test performed after an incubation period of 20 days. Typical response to 7,000 units in 100 cc oxygenated Ringer-Locke solution. Time in 30 seconds.

[4] Lyons, C., *J. A. M. A.*, 1943, **123**, 1007.

TABLE II.
Anaphylactic Response of the Isolated Uterus to Penicillin. Both Cornua Used.

No.	Sensitizing dose, units	Incubation period, days	Shocking dose units	Response	Desensitization
1	4 x 500 + 1 x 1000 s.c.	16	(a) 3000	None	
			(b) 7000	"	
2	5 x 500 i.p.	19	(a) 3000	"	
			(b) 7000	Positive delayed	
3	" "	20	(a) 7000	"	Ineffective
			(b) 2000	" delayed	"
4	" "	21	(a) 2000	None*	
			(b) 2000	"	
5	" s.c.	23	(a) 2000	"	
			(b) 2000	"	
6	" "	25	(a) 8000	" *	
			(b) 15000	" *	
7†	" i.p.	29	(a) 15000	Positive delayed	"
			(b) 15000	None	
8†	4 x 500 + 1 x 1000 i.p.	31	(a) 15000	"	
			(b) 15000	"	
9†	" "	32	(a) 3000	"	
			(b) 3000	Doubtful	
10†	5 x 500 s.c.	34	(a) 20000	None	
			(b) 20000	Positive delayed	Effective
11†	4 x 500 + 1 x 1000 s.c.	35	(a) 20000	None	
			(b) 20000	"	
12†	5 x 500 s.c.	36	(a) 20000	Positive delayed	"
			(b) 20000	None	
12	1 x 50000 i.v.	13	(a) 20000	"	
			(b) 20000	"	

* Response to standard dose posterior pituitary extract (0.12 mg) subnormal.
† Also sensitized to horse serum with typical response including desensitization.

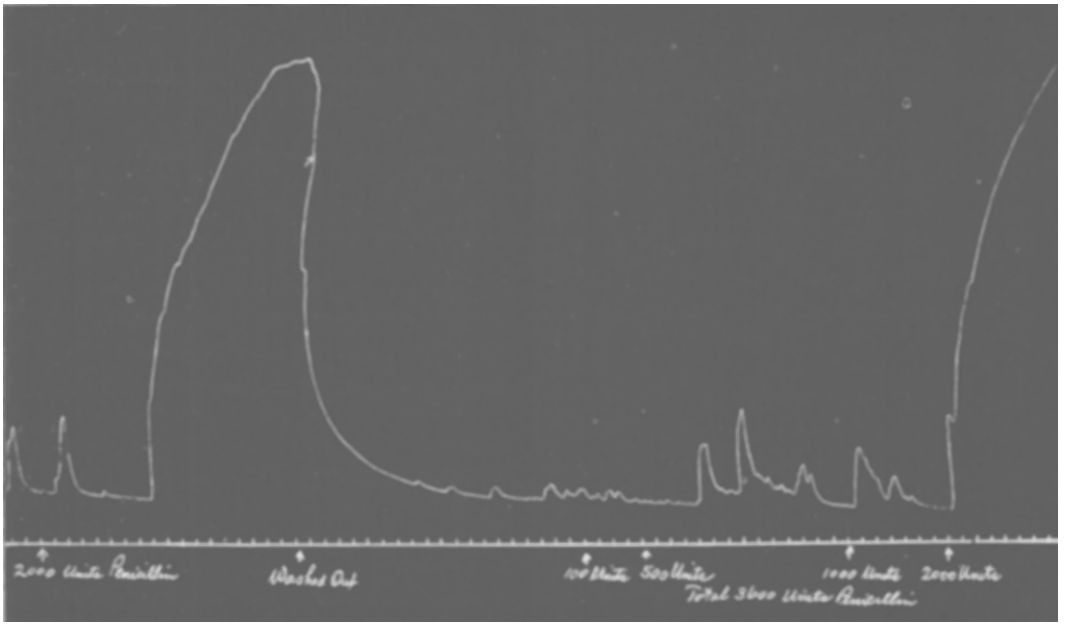

FIG. 2.
Second uterine cornu of same animal as described in Fig. 1. Note delayed reaction, and ineffective desensitization.

tions, though of infrequent occurrence, prompted a study of the substance in guinea pigs to ascertain whether or not it would be possible to demonstrate anaphylactic reactions by the usual methods of sensitization. The primary toxicity of penicillin in guinea pigs was studied by Hamre and associates[5] who showed that the acute fatal dose injected intravenously is upwards of 50,000 units per kg while 7,000 to 12,000 units per kg given daily by the subcutaneous route cause death in several days.

In the present experiments 2 series of guinea pigs were sensitized by daily subcutaneous or intraperitoneal injections of penicillin over a period of 5 days; one group to be used for intracardiac or intravenous injection of the antigen after an adequate incubation period, and the other for tests on the isolated uterus by the Schultz-Dale technique. The individual sensitizing doses ranged from 300 to 1,000 Oxford units and the shocking doses ranged from 5,000 to 23,000 units in the intracardiac group and from 2,000 to 20,000 units in the isolated uterus group. Some of the guinea pigs in each group were also sensitized to horse serum, the object being to ascertain the extent of anaphylactic state in the animals in the event that the penicillin reactions were negative. The sensitizing dose of horse serum was uniformly 0.1 cc and the magnitude of the shocking dose varied as shown in the tables. In the latter group one animal was sensitized by a single intravenous injection of 50,000 Oxford units.

The results of these tests are shown in two tables. Table I gives a detailed account of 11 animals tested after a suitable incubation period by the intravenous or intracardiac route. Of these, 2 died with typical symptoms of anaphylactic shock and 2 showed moderate to severe symptoms followed by recovery. Five in this group were refractory and 2 showed mild symptoms of a doubtful nature. It is to be noted, however, that of the 7 animals refractory to penicillin 3 were also wholly refractory to horse serum and 2 though responding with typical symptoms to the horse serum made good recoveries. The shocking dose of horse serum was injected in the same manner as the shocking dose of penicillin.

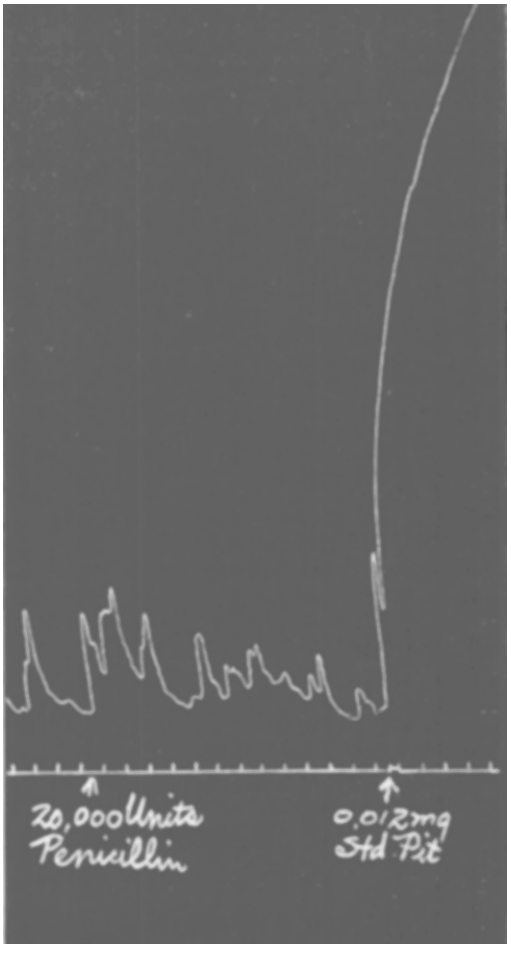

FIG. 3.
Shows negative response to 20,000 units in a guinea pig sensitized by 4 daily doses of 500 units and 1 of 1,000 units injected subcutaneously, and tested after an incubation period of 35 days. The response to a test dose of pituitary was normal.

In Table II are given the results with the isolated uterus method. In this there were 13 animals, each uterine cornu having been subjected to the test, making a total of 26 tests. A positive response was elicited in 6 preparations obtained from 5 animals. The preparations of 7 animals gave negative results, and one doubtful. The physiological condition of the uterine horn was tested in each case by a small dose of posterior pituitary extract, and

[5] Hamre, D. M., Rake, G., McKee, C. M., and MacPhillamy, H. B., *Am. J. Med. Sci.*, 1943, **206**, 642.

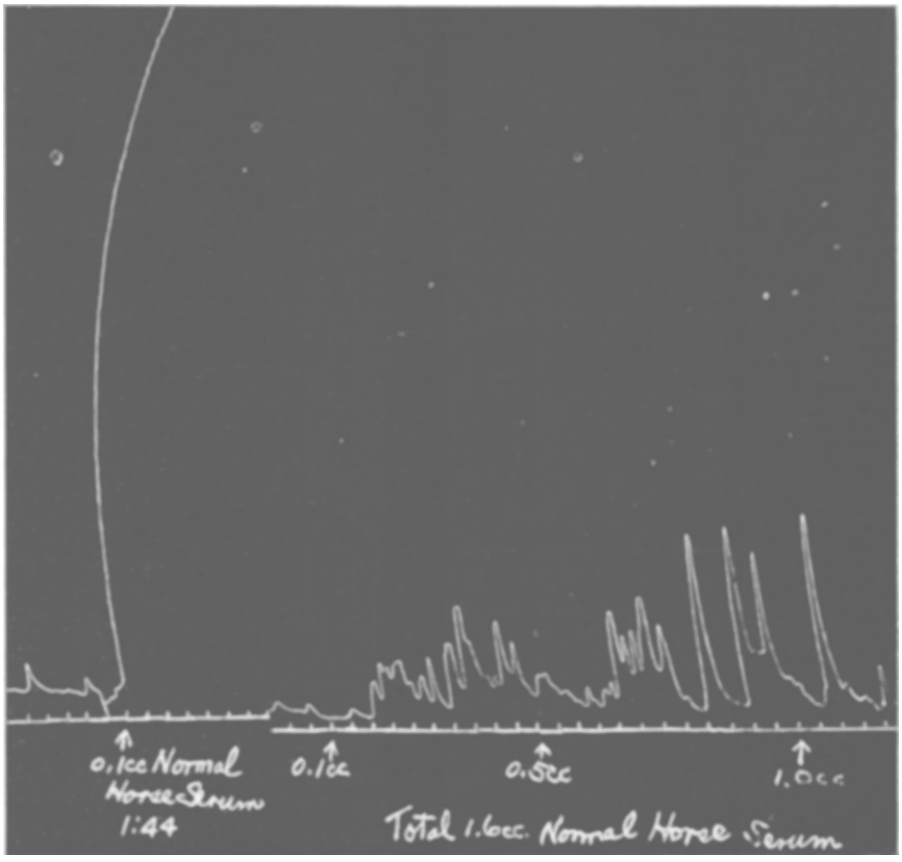

FIG. 4.
Same preparation as shown in Fig. 3. Note typical response to horse serum and characteristic desensitization.

in 6 of the experiments which had been sensitized to horse serum as well a typical response was produced by the latter antigen. In those experiments in which a positive response was obtained to penicillin desensitization was attempted but it frequently was ineffective. Moreover, the penicillin response when present was delayed, all of which indicates that while sensitization to penicillin is possible it is not uniform and when present is atypical.

In a series of 8 normal guinea pigs under the same experimental conditions penicillin had no appreciable effect on the contractions of the isolated uterus in doses of from 20,000 to 30,000 units. Each uterine horn was tested separately, making a total of 16 tests.

Comment. The present experiments indicate that anaphylactic sensitization in the susceptible guinea pig can be achieved with the penicillin as currently prepared and marketed. The penicillin used in this work consisted of several commercial lots of the sodium salt manufactured by at least 5 different distributors, and was of the same degree of purity as that used currently in the clinic. To what extent impurities alone may have been responsible for some of our results cannot be stated at present. If the reactions observed were due to impurities it is obvious that they are inherent in the commercial products in which they are inseparable from the active substance. Parallel experiments with the purest obtainable crystalline material, or preferably synthetic penicillin, would determine this point. In any event, this work appears to afford at least a partial explanation for some of the untoward effects reported in the clinical use of the drug as supplied commercially. It should also be

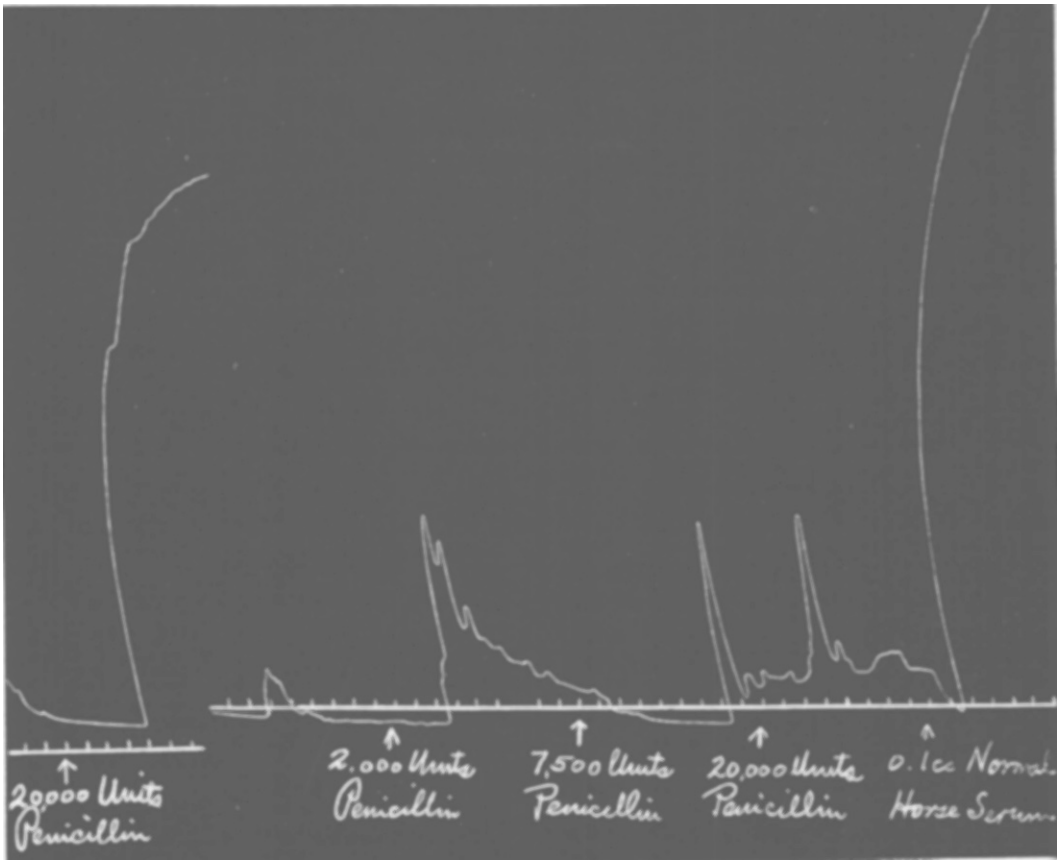

FIG. 5.

Guinea pig sensitized to penicillin and horse serum. Tested for sensitization against both antigens after an incubation period of 36 days. Note prompt response to horse serum and positive though delayed reaction to penicillin with effective desensitization.

pointed out that the positive reactions are for the most part atypical, in that the reactions are often delayed, and desensitization often ineffective. It would seem that the penicillin (commercial) antibody-antigen combination lacks permanency and is more readily reversible than is the case in anaphylactic reactions following sensitization with true proteins. The incomplete and imperfect sensitization with penicillin may perhaps be explained on the supposition that the precipitin-antigen union in this case results in too rapid flocculation to effect cell permeability (Dale[6]). The imperfect antibody-antigen union would also have the effect of failing to saturate or exhaust anaphylactic antibodies, in this case the smooth muscle receptors, which according to Weil and Coca[7] is an essential factor in the mechanism of desensitization.

Summary and Conclusions. Tests for anaphylactic sensitization with penicillin were attempted by the isolated uterus technique and by intracardiac or intravenous reinjection of antigen. Sensitization with penicillin as currently prepared and marketed was demonstrated by both methods. Sensitization was not uniform, and usually atypical in that the uterine response was often delayed and desensitization often difficult to demonstrate. The results appear to afford a partial explanation for some of the reactions encountered in the clinical use of the drug.

[6] Dale, H. H., *Bull. Johns Hopkins Hospital*, 1920, **31**, 310.

[7] Weil, R., and Coca, A. F., *Z. Imm.*, 1913, **17**, 141.