carboxylation is associated with phosphorylation of adenylic acid to yield adenosine triphosphate. Nachmansohn and Machado[6] have shown that adenosine triphosphate intensifies the activity of an enzyme system (choline acetylase) which produces the final synthesis of the cholinergic transmitter. Our knowledge about this enzyme is still rudimentary, but if the enzyme should prove specific, the disintegration of pyruvic acid initiated by cocarboxylase should furnish the system with 2 important elements, *viz.*, acetic acid and adenosine triphosphate, concerned with the synthesis of acetylcholine from choline.

[6] Nachmansohn, D., and Machado, A. L., *J. Neurophysiol.*, 1943, **6**, 397.

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Effect of Pemphigus Serum on the Behavior of Rats.

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Macht and Pels,[1] by employing special phytopharmacological methods have demonstrated the presence of a toxic substance in the blood of pemphigus patients. This toxic reaction is not given by other dermatoses so that the phytopharmacological test has been found useful in establishing the diagnosis of pemphigus at an early stage and in doubtful cases.[2-4] More recently, Macht and Ostro have extended our knowledge concerning this substance and have employed the test in evaluating therapeutic procedures employed in treating this disease.[5] From these experiments it was evident that pemphigus is not a simple dermatosis but is primarily a constitutional or systemic disease or a form of toxemia. Concomitant with the characteristic skin eruptions the patient exhibits profound disturbances of a systemic nature, the most prominent symptoms of which come from the nervous system. This has been noted already 50 years ago by Payne[6] who, in his classical lecture on pemphigus, stated:

"If any system of the body is affected besides the skin it seems to be the nervous system rather than any other. Obscure symptoms of nervous depression are sometimes recognized before the eruption begins, and in the severer forms the cerebral nervous system—or at least the brain—is evidently profoundly affected, as is shown by delirium and nervous prostration."

In order to investigate further the effects of this toxic substance in pemphigus serum on the nervous system the present writer undertook experiments on the behavior of white rats by 3 kinds of experimental psychological methods. First, a study was made on the behavior of the rats in Watson's circular maze. While views differ as to the complete significance of the process through which the rats undergo the learning of the maze problem, it is generally agreed that such a performance involves not only a response to the proprio-sensitive stimuli but also a *choice* reaction or faculty of *discrimination*, which the rats exhibit under the urge of hunger. A second series of experiments was made on rats which have been trained to walk a tightly stretched rope 8 feet long from one platform to another on which food had been placed. This performance involves a delicate neuro-muscular coordination. In the third series of experiments, rats were trained to climb a tightly stretched per-

[1] Macht, D. I., and Pels, I. R., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 237.

[2] Pels, I. R., and Macht, D. I., *Arch. Dermat. and Syph.*, 1929, **19**, 640.

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[5] Macht, D. I., and Ostro, M., *Urologic and Cutaneous Rev.*, 1946, **19**, 271.

[6] Payne, J. F., *Lancet*, 1893, **2**, 421.

TABLE I.
Influence of Sera on Behavior of Rats.

Exp. No.	Wt of rat g	Method of inj.	Kind of serum inj.	Dose cc	Phyto-toxic index %	Behavior before inj.			Behavior 30 min. after inj.			Behavior 2 hr later			Remarks
						In maze sec.	On rope sec.	Climbing sec.	In maze	On rope sec.	Climbing sec.	In maze	On rope sec.	Climbing sec.	
I	250	Periton.	*Pemph. vulg.*	0.5	55	6.4			Depressed stalled			Completely stalled			Marked depression
II	"	Subcut.	" "	0.5	54	12.0 1 error			Completely stalled			Completely stalled			Marked dep.
III	"	Periton.	" "*	0.5	43	7.8			Stalled depressed			Marked depress.			Dies overnight
IV	"	"	" *foli.*	0.5	45	7.5			Marked depress.			Depressed stalled			" late same day
V	150	Subcut.	" "	0.3	45	9.0	3.5	4.3	9.1 sec.	3.5	5.5	20.3 sec.	6.1	8.7	Dep. min. dose
VI	"	"	" *vulg.*	1.0	56	7.6	4.9	11.2	10.8 "	4.5	13.2	10.0 "	4.7	13.1	Dep.
VII	"	Periton.	" "	1.0	43	6.1	3.3	8.0	10.4 "	4.7	15.3	11.8 "	6.2	10.0	"
VIII	"	"	" "	0.5	52	7.7	5.6	9.5	35.7 " 2 errors	5.5	11.9	10.2 " 1 error	5.2	15.5	Marked dep.
IX	"	Subcut.	" "	0.6	52	7.7	6.2	12.6	10.5 sec.	8.3	10.0	8.0 sec.	5.4	10.0	Dep.
X	"	"	" *foli.*	1.0	59	13.1 6 errors	5.0	7.0	24.9 " 8 errors	4.8	7.7	25.6 " 5 errors	6.3	9.9	Great dep. next day
XI	"	Periton.	" "	1.0	59	6.2	3.9	5.7	Prostrated	Pros.	Pros.	Pros.	Pros.	Pros.	Dies overnight
XII	"	Subcut.	" *vulg.*	1.0	55	7.3	4.1	9.8	10.5 sec.	6.8	19.1	13.3 sec.	Stalled Depressed	21.7	Marked dep.
XIII	"	Periton.	" " during remission	1.0	60	6.3	3.6	6.2	8.1 "	4.9	9.0	6.0 "	4.5	8.0	Mild dep. during remission period
XIV	"	Subcut.	*Pemph. foli.*	0.3	58	12.5 2 errors	4.1	5.2	10.0 " 1 error	3.9	8.1	17.3 " 2 errors	4.3	7.9	Mild dep. from minute dose
XV	"	"	" "	0.4	58	6.2	4.3	12.8	6.4 sec.	4.3	10.5	9.0 sec.	5.0	11.6	Mild dep.
A	250	Periton.	Normal human	0.5	72	7.5			7.9 "			7.2 "			Control sera
B	"	"	" "	0.5	70	6.7			6.4 "			6.1 "			" "
C	"	Subcut.	" "	1.0	75	5.8			6.1 "			5.4 "			" "
D	"	Periton.	Saline	4.0	100	6.8			6.9 "			6.4 "			Physiol. saline
E	150	"	Pernicious anemia	0.5	52	8.9	4.9	4.9	7.7 "	4.5	5.8	8.3 "	3.4	4.1	Control pern. anemia
F	"	Subcut.	Normal human	0.5	70	5.1	3.7	7.5	5.2 "	3.9	7.7	5.1 "	3.7	8.0	Control
G	"	"	" "	1.0	70	6.0	5.1	14.2	6.3 "	5.1	14.7	6.0 "	5.4	14.4	"
H	"	"	" "	0.5	72	5.1	3.7	5.0	4.9 "	2.7	3.3	4.9 "	3.3	5.2	"
I	"	"	" "	0.5	73	5.1 2 errors	3.2	4.3	8.2 " 2 errors	3.2	4.1	5.6 " 2 errors	3.3	4.3	"

* Mouth

pendicular rope. This exercise also involves neuro-muscular coordination but is especially designed for testing the work capacity of the skeletal muscles. A detailed description of the methods employed is given by the senior author elsewhere.[7]

Fifteen experiments were performed with injections of small amounts of pemphigus serum into trained rats and an equal number of experiments were performed with normal and other control sera. For obvious reasons it was not advisable to employ the same rats too frequently because of the danger of anaphylactic reaction. In results reported here no allergic reactions were elicited. Two groups of white rats were employed. One group consisted of large-sized male rats each weighing approximately 250 g. The other and larger group consisted of younger white male rats each weighing approximately 150 g. The larger animals were trained only in the circular maze whereas the smaller and lighter rats were trained by all the 3 methods described above. The injections were made in some cases intraperitoneally while subcutaneous injections were made in other cases. Table I gives the results obtained in the 15 experiments with pemphigus serum and also in 9 control experiments. It will be noted that in one of the controls an injection of physiological saline alone was given, while in another control experiment, an injection was given of the blood serum from a case of pernicious anemia which, as was shown by one of the authors, is toxic for living plants.[8] Pernicious anemia blood, however, is not depressant for rats. Table I gives the weight of the rats, the method of injection, the dose of serum injected, the phytotoxic index of the sera and the performance of the animals before injection and after injection. In every case the running time in the maze is an average of 3 runs and when the animals made mistakes by running into blind alleys the number of such errors is noted. In the experiments on rope walking and rope climbing the time in seconds is given also as an average of 3 runs on the horizontal rope and 2 climbs on the vertical rope.

By the phytotoxic index in Table I is meant the ratio of the growth of the seedling roots in 1% serum solution to its growth in the normal control solution according to the formula,

$$\text{Phytotoxic index of growth} = \frac{X}{N} \times 100$$

in which N represents the average growth of controls and X the average growth in the 1% serum solution.

Results. It was noted that in the control experiments neither the injections of physiological saline nor of normal blood serum produced much depression or any other effect on the behavior of the rats. On the other hand, pemphigus sera produced a definite depression and in some cases a very marked prostration. This effect was noted not only in the maze experiments which indicate the influence of the toxic constituent on the "intelligence" of the animals but also on the neuro-muscular coordination as indicated by the running on the horizontal rope and especially in a weakening of the muscles as revealed in the climbing experiments. Two kinds of pemphigus sera were examined. In the majority of the experiments, serum from cases of pemphigus vulgaris was injected, but in several of the other experiments the serum from 2 cases of pemphigus foliaceus was injected. Two rats, one large one and one small one, died after injections of the latter variety.

The toxic effects obtained in this investigation throw a light on the profound constitutional symptoms of patients afflicted with pemphigus and stress the fact that the cutaneous and mucous membrane lesions are but the external manifestations of a grave systemic disturbance which so far as is known is not a bacterial infection but which is definitely characteristic of a toxemia, as has been proven by pharmacological experiments. It has been claimed that in the late stages of pemphigus there is an increased amount of organic phosphorus and a reduced calcium content of the blood. The senior author, in many previous experiments with such blood sera and with sera from cases of inorganic acidosis, did not obtain such depressant effects

[7] Macht, D. I., *Exp. Med. and Surg.*, 1943, **1**, 260.

[8] Macht, D. I., *J. Pharm. and Exp. Therap.*, 1926, **29**, 461.

on the central nervous system after such small doses of serum, as were obtained in the present research. In the opinion of the authors, pemphigus should be described in treatises on internal diseases with equal propriety as smallpox, leprosy, syphilis, pellagra, and other constitutional diseases are discussed in such text books.

Summary. 1. The neuro-muscular responses and general behavior of white rats were studied, (1) in a circular maze, (2) on animals trained to walk a tightly-stretched horizontal rope, and (3) on animals trained to climb a vertically-stretched rope.

2. Injections of small quantities of pemphigus sera in these rats produced a definite depression of performance in all 3 tests, while control experiments with normal sera did not affect the rats adversely.

3. These findings corroborate the authors' experiments with pemphigus sera on plants and indicate that this disease is a systemic, rather than a cutaneous one, as is indicated by the toxic effects exerted by the blood serum of patients with pemphigus.

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The Vitamin B_6 Group. IX. Comparative Growth and Anti-anemic Potencies of Pyridoxal, Pyridoxamine and Pyridoxine for Dogs.*

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Fouts *et al.*[1,2] and subsequently others[3,4] showed that dogs, when kept on vitamin B_6 deficient diets, developed a characteristic microcytic hypochromic anemia which could be cured with pyridoxine. Anemia has also been produced in chicks,[5] ducks[6] and swine[7] by feeding diets low in vitamin B_6.

Since enzymatically effective derivatives of vitamin B_6 are related directly to pyridoxal[8,9] and pyridoxamine,[10] rather than to pyridoxine, and since the 3 compounds have equivalent growth-promoting activities when injected intraperitoneally into rats or chicks,[11] it seemed possible that the antianemic potencies of the former 2 compounds might surpass that of pyridoxine. Results reported below show that when injected intravenously, pyridoxal, pyridoxamine and pyridoxine are equally active in increasing hemoglobin levels and supporting growth of dogs.

Experimental. Littermate male pups 8 weeks old (No. 41 and 42) and one older female pup 14 weeks old (No. 40) were treated twice with an anthelmintic (tetrachlorethylene), freed of external parasites with rotenone and placed in individual cages. The vitamin B_6-deficient diet had the following

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For paper VIII of this series see reference 11.

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