by Marshall, Litchfield, and White,[15] and by Seeler, Graessale, and Dusenberg.[16] PABA counteracts the inhibiting effect of sulfanilamide on cell division of the flagellate *Polytomella caeca*.[17] Similar antagonism is exerted by para-aminobenzoic acid on fresh water diatoms;[18] on pigment formation of *Pseudomonas seruginosa*;[19] on the effect of sulfanilamide on experimental *Lymphogranuloma venereum* virus,[20] and on some yeasts.[21]

It is therefore surprising that the influence of sulfanilamide and PABA combinations on higher plants such as *Lupinus albus* is diametrically opposite and the converse of antagonism.

This is of interest from the standpoint of general biology. In the earlier days of bacteriology it was generally held that bacteria are microscopic plants. In fact, older writers often resorted to yeast cultures for testing the antiseptic properties of drugs. In the light of our present findings the behavior of bacteria under the combined action of sulfanilamide and PABA resembles more the effects produced by these drugs on plasmodia and flagellates which are commonly classed as animal organisms. The results yielded by a study of *Lupinus albus* emphasize the uncertain demarcation between the lower animal and plant organisms. The present findings illustrate well the dangers of hasty and sweeping generalizations in pharmacology. It is hazardous to apply deductions from data of one species of zoo- or phyto-pharmacological test-objects to another, without experimental tests in support of such conclusions.

Summary. Pharmacological studies on root-growth of *Lupinus albus* seedlings under standardized and rigidly controlled conditions reveal that: (1) Of the 3 amino-benzoic acids the *meta* isomer is the most toxic, and the *para* isomer is the least toxic. (2) Para-aminobenzoic acid exerts a diphasic effect on root-growth: Very weak dilutions are growth-promoting, while stronger concentrations are growth-inhibitory. (3) Sulfanilamide also exhibits a similar action, some concentrations inhibiting growth while other concentrations promote it. (4) Similar findings were made with 6 other sulfonamides which were examined. (5) Combinations of sulfanilamide and para-aminobenzoic acid in every case exerted not an antagonistic, but on the contrary, a synergistic or potentiated depression on growth of *Lupinus albus* roots.

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Cause of an Outbreak of Encephalitis Established by Means of Complement-Fixation Tests.*

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On 16 July, 1945, members of the staff of Naval Medical Research Unit No. 2 arrived on Okinawa to study an outbreak of encephalitis which had appeared among natives of the island during the first week in July. The danger that the encephalitis might interfere with military and naval operations in the Ryukyus made it imperative that the etiologic agent be identified as soon as possible so that proper control measures could be instituted.

* The Bureau of Medicine and Surgery of the Navy does not necessarily endorse the views set forth in this paper.

Because of the proximity of Okinawa to Japan, and because the disease had appeared in midsummer, it was thought that the active agent most likely to be involved was Japanese B encephalitis virus. It was believed that an attempt to demonstrate the development during convalescence of complement-fixing antibodies against this and other neurotropic viruses offered the best means of securing prompt identification of the virus responsible for the outbreak. Accordingly, acute and convalescent sera from patients ill with encephalitis on Okinawa were sent to the Guam laboratories of Naval Medical Research Unit No. 2 where they were tested by the method described by Casals[1] for the presence of complement-fixing antibody against Japanese B and other neurotropic viruses. On July 23, 2 specimens of serum from each of 3 Okinawan patients reached Guam and were tested the following day. Both specimens from 2 of these patients (No. 1 and No. 3) were negative. Serum of the third patient (No. 2) obtained on the eighth day of disease was negative but serum obtained 14 days after he became ill was positive for complement-fixing antibody against the Japanese B virus in a final dilution of 1:16. This indicated that the patient had developed antibodies against this virus as the result of his illness, and that the disease from which he was convalescing was caused by Japanese B virus. It was, therefore, demonstrated within 24 hours after the first samples of serum reached the laboratory that Japanese B virus was present on Okinawa and was responsible for at least a portion of the encephalitis occurring there. Subsequent complement-fixation tests carried out with sera obtained from 17 additional patients on Okinawa who were believed to be suffering from encephalitis have established beyond doubt that a number of them were recovering from infection with this virus.

Results of complement-fixation tests carried out with sera from patients on Okinawa are summarized in Table I. Examination of this table reveals that 6 patients (No. 2, No. 19, No. 30, No. 31, No. 36, and No. 61) showed no complement-fixing antibody against Japanese B virus early in their illness, but developed a significant titer against this active agent between the 14th and 27th day of disease. A 2-plus or greater reaction in a final serum dilution of 1:8 is significant, and these 6 patients developed a titer of 1:16 or greater. It is certain, therefore, that they were convalescent from Japanese B encephalitis. Results obtained with early and convalescent sera from a second group of 5 patients (No. 6, No. 7, No. 28, No. 33, and T.W.H., an American soldier) showed that these persons had developed a significant increase in titer of antibody against Japanese B virus during the time which elapsed between the collection of the first and second specimens of their sera. It is practically certain that these patients also had suffered infection with Japanese B virus.

It is also shown in Table I that sera obtained from patients No. 15, No. 16, and No. 52 were positive 12 to 14 days after they became ill, but that no increase in complement-fixing antibody was observed during the next 8 to 15 days. The significance of the findings in this group is not clear, and is open to several interpretations which will not be discussed here. Only sera drawn late in convalescence from patients H-4 and No. 41 were available for study. The high titer of antibody in their sera indicates that they probably had had recent experience with Japanese B virus, but the relation of this antibody to their illness cannot be determined with certainty.

An examination of Table I also reveals that all sera of patients No. 1, No. 3, No. 27, and No. 39 were negative, indicating that their illness was not Japanese B encephalitis, or that if they had been infected with this virus they had failed to produce antibody against it. It is of interest in this connection to note that autopsy of patient No. 3 proved that his central nervous system symptoms were due to tuberculous meningitis.

It is known that laboratory animals hyperimmunized by vaccination with Japanese B virus develop a high titer of complement-fixing antibody against this virus and a low titer against the virus of St. Louis encephalitis.[2]

[1] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

[2] Casals, J., *J. Exp. Med.*, 1944, **79**, 341.

TABLE I.

Summary of Complement-Fixation Tests with Sera of Patients on Okinawa Showing Symptoms of Encephalitis.

Date of test	Case	Day of disease serum obtained	Japanese B virus antigen Final serum dilution 1 to: 4	8	16	32	64	128	Western equine Virus antigen Final serum dilution 1 to: 4	8	16	32	64	128	St. Louis Virus antigen Final serum dilution 1 to: 4	8	16	32	64	128	Serum Control
7/24	2	8	0	0	0	0	0														0
24	2	14	4+	3+	2+	±	0														0
8/ 4	2	8	0	0	0	0	0														0
4	2	21	3+	3+	2+	±	0		0	0	0	0	0		0	0	0	0			0
19	30	4	0	0	0	0															0
19	30	20	4+	4+	3+	±	0	0	0	0	0	0	0								0
19	19	10	0	0	0	0															0
19	19	27	4+	4+	4+	3+	±	±	0	0	0	0	0								0
19	31	4	0	0	0	0															0
19	31	19	4+	4+	4+	+	±	0	0	0	0	0	0								0
9/12	36	3	0	0	0	0	0	0													0
12	36	32	4+	4+	4+	3+	0	0	0	0	0	0	0	0							0
12	61	3	±	±	0	0	0	0	±	0	0	0	0	0							±
12	61	31	4+	4+	4+	4+	4+	3+	0	0	0	0	0	0							0
8/19	33	2	2+	±	0	0															0
19	33	17	4+	4+	4+	3+	2+	±	0	0	0	0	0								0
19	28	11	3+	±	±	0	0														0
19	28	26	4+	4+	3+	3+	0	0	±	0	0	0	0	0							0
19	T.W.H.	5	4+	3+	±	0															0
19	T.W.H.	18	4+	4+	4+	4+	4+	3+	0	0	0	0	0								0
4	7	12	3+	3+	2+	+	±								0	0	0	0	0		0
4	7	19	4+	4+	4+	3+	2+	+	0	0	0	0	0	0	0	0	0	0	0		0
19	6	11	4+	4+	4+	+	±														0
19	6	26	4+	4+	4+	4+	2+	±	0	0	0	0	0								0
19	15	14	4+	2+	+	±	0														0
19	15	29	3+	3+	+	±	0		0	0	0	0	0								0
19	52	12	4+	2+	+	±	±														0
19	52	20	3+	2+	+	0	0		±	0	0	0	0								0
19	16	13	3+	2+	±	±	0														0
19	16	29	3+	2+	±	±	±		±	0	0	0	0								0
7/31	H-4	24	4+	4+	2+	±	±	0													0
8/ 4	41	22	4+	4+	4+	4+	3+	3+	0	0	0	0	0	0	0	0	0	0	0	0	0
7/24	1	8	0	0	0	0	0														0
24	1	14	0	0	0	0	0														0
8/ 4	1	8	0	0	0	0	0	0	0	0	0	0	0	0							0
4	1	14	0	0	0	0	0	0	0	0	0	0	0	0							0

7/24	3	8	0	0	0	0	0	0																0
24	3	14	0	0	0	0	0	0																0
8/19	27	8	0	0	0	0																		0
19	27	24	0	0	0	0	0	0	0	0	0	0	0	0										0
7/31	39	20	+	±	0	0	0	0																0
24	Immune Jap. B Hamster (Pool 1)		4+	4+	4+	2+	±																	0
31	Immune Jap. B Hamster (Pool 2)		4+	4+	4+	4+																		
8/ 4	Immune Jap. B Hamster (Pool 2)			4+	4+	4+											0	0	0					0
19	Immune Jap. B Hamster (Pool 3)		4+	4+	4+	4+	4+	±	0	0	0	0	0											0
9/12	Immune Jap. B Hamster (Pool 2)		4+	4+	4+	4+	4+	2+	3+	0	0	0	0	0										0
8/ 4	Immune St. Louis Mouse		±	0	0												4+	4+	4+	4+				0
4	Immune West. Equine G.P.								4+	4+	4+	4+	4+	3+			±	0	0	0				0
19	Immune West. Equine G.P.		+	0	0	0	0		4+	4+	4+	4+	4+											0
9/12	Immune West. Equine G.P.		2+	±	±	0	0	0	4+	4+	4+	4+	4+	4+										0

TABLE II.

Identification by Complement Fixation Reaction of Virus Isolated on Okinawa from Patient Suffering from Encephalitis.

Serum	Nakayama strain Jap. B antigen (Lyophilized) Final serum dilution						Okinawa No. 53 antigen (Freshly prepared) Final serum dilution						Normal mouse brain antigen (Freshly prepared) Final serum dilution						Western equine antigen (Lyophilized) Final serum dilution						Serum Control
	1 to:						1 to:						1 to:						1 to:						
	4	8	16	32	64	128	4	8	16	32	64	128	4	8	16	32	64	128	4	8	16	32	64	128	
Hamster—(Pool No. 1) vaccinated with Nakayama Jap. B virus	4+	4+	3+	±	±	±	4+	4+	4+	3+	+	±	0	0	0	0	0	0	0	0	0	0	0	0	0
Hamster—(Pool No. 2) vaccinated with Nakayama Jap. B virus	4+	4+	3+	±	±	±	4+	4+	4+	3+	±	±	0	0	0	0	0	0	0	0	0	0	0	0	0
Guinea pig vaccinated with West. equine virus	±	±	0	0	0	0	±	±	0	0	0	0	±	±	±	0	0	0	4+	4+	4+	4+	4+	4+	0

Heretofore, no sera from persons convalescing from an infection with Japanese B virus have been available for study by means of complement-fixation tests, so that it was not known whether a similar cross antibody reaction occurs in the sera of human beings. Table I shows that sera from 3 convalescent persons which fixed complement in the presence of Japanese B virus failed to do so with St. Louis virus. Furthermore, 15 sera which contained complement-fixing antibody against Japanese B virus showed no antibody against the virus of Western equine encephalitis. Two sera which were positive against Japanese B virus were negative against lymphocytic choriomeningitis virus, and 2 other such sera were negative against Eastern equine encephalitis.

The identification of Japanese B virus as the causative agent of the Okinawan outbreak of encephalitis by means of the serologic evidence described above was confirmed by the isolation of a virus from the brain of a patient (No. 53) dying of encephalitis. This virus was isolated by a group of medical officers of the U. S. Army and Navy (two of the authors of the present communication, L.T. and J.P., were in this group). A sample of this agent was sent to our laboratory on Guam. An antigen suitable for complement-fixation tests was prepared from brains of mice dying of infection with the third passage of this virus. As shown in Table II, the Okinawa virus antigen fixed complement in the presence of sera of hamsters immunized against the Nakayama strain of Japanese B virus, but no significant complement-fixation occurred when it was tested against serum of guinea pigs immunized against Western equine encephalitis virus. The virus isolated on Okinawa was, therefore, shown to be Japanese B virus by means of its specific complement-fixation reaction.

Summary. 1. By complement-fixation tests carried out with human sera, an outbreak of encephalitis which began on Okinawa in July, 1945, was promptly shown to be due to Japanese B virus. 2. The tests showed that at least 11 of 20 patients studied had suffered an attack of encephalitis caused by this virus. 3. None of 15 human sera which were positive against Japanese B virus showed complement-fixing antibodies against Western equine encephalitis virus, and a few such sera were negative when tested against the viruses of Eastern equine encephalitis, St. Louis encephalitis, and lymphocytic choriomeningitis. 4. A virus isolated from a patient on Okinawa suffering from encephalitis was identified by complement-fixation reactions as Japanese B virus.

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Observations on Antimicrobial Action of 2, 3-Dichloro-1, 4-Naphthoquinone, and its Reversal by Vitamins K.

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Since Ter Horst and Felix[1] have found a marked antifungal action of 2,3-dichloro-1,4-naphthoquinone, the idea occurred that this effect might be linked with the structural similarity of the compound to vitamin K, much as that of the sulfonamides is related to their analogy with the metabolite *p*-aminobenzoic acid. This view seemed probable since Kuhn *et al.*[2] had shown previously that 6,7-dichloro-9-ribityl-isoalloxazine had antimicrobial action which was reversible by riboflavin. The change involved in passing from the vitamin riboflavin to the inhibitor 6,7-dichloro-9-ribityl-isoalloxazine was the replacement of 2 methyl groups attached to

* With the technical assistance of M. L. Collyer.

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