EFFECT OF ENZYME INHIBITORS AND ACTIVATORS ON THE MULTIPLICATION OF TYPHUS RICKETTSIAE

I. Penicillin, Para-Aminobenzoic Acid, Sodium Fluoride, and Vitamins of the B Group*

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INTRODUCTION

Typhus rickettsiae, like the viruses, are obligate intracellular parasites, multiplying freely within their host cells when conditions exist which are favorable for their growth. It has been recognized for many years that favorable conditions for rickettsial growth are associated with a low rate of metabolic activity in the host cells (1, 2). Many viruses, on the other hand, particularly those of relatively small size, grow most freely in cells which are metabolizing actively (2). Riboflavin deficiency, which slows down cell metabolism by interfering with respiration, has been shown to be effective in bringing about conditions favorable for rickettsial growth in rats (3). On the other hand, riboflavin deficiency (4) and thiamin deficiency (5) protect mice to some extent against poliomyelitis (a small virus), significantly reducing the mortality from this infection.

By studying the effect of various enzyme inhibitors and activators on the intracellular multiplication of rickettsiae, it seems possible that information can be obtained concerning the specific enzyme systems involved in rickettsial metabolism. The enzyme activators to be employed will include many agents classed as vitamins as well as a variety of endocrine products. Enzyme inhibitors include chemotherapeutic agents, and many organic and inorganic chemicals which are known to interrupt or modify cellular metabolism.

In some instances the precise mode of action of the agent is known, while in other instances exact information is lacking. Although the intimate metabolism of living cells is in general incompletely understood, many specific facts have been established which, it is believed, can be applied advantageously to the solution of the problems of intracellular parasitism. It also seems reasonable to expect that knowledge of the enzyme systems themselves will be advanced by studies of this type.

In this report, the positive results obtained thus far will be presented in

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detail. Those agents which have shown no definite effect on the multiplication of rickettsiae will be mentioned briefly, since negative results are of some interest in this type of study.

Material and Methods

A murine strain of typhus, in its 30th passage in fertile eggs was used. This strain was originally isolated in Mexico. Its virulence for guinea pigs has remained unchanged in spite of 35 passages through eggs. In its earlier passages, this strain was used for testing the chemotherapeutic effectiveness of penicillin in the yolk sac (6) and in mice (9).

The technic of egg injection was essentially that used in previous experiments (6), with certain modifications which have been found to save much time and almost to eliminate bacterial contamination. When the control eggs in a previous series begin to die with heavy rickettsial infection (usually between the 6th and 8th days after injection), an egg in this series, preferably one with its embryo still active, is chosen as the source of inoculum. The end of the egg containing the air sac is swabbed with a 4 per cent alcoholic solution of iodine, and the shell is removed piecemeal with sterile forceps. When the opening is sufficiently large, the inner air sac membrane is stripped away and the entire contents of the egg shell are allowed to slide out into a pyrex dish 5 cm. in height and 9 cm. in diameter across the top (the commercial pyrex custard dish). The dish previously has been sterilized with alcohol and flame and covered with the top half of a sterile petri dish.

The yolk sac membrane is grasped with sterile forceps and a segment estimated to weigh about 1 gm. is snipped off with curved scissors, and placed in the chamber of a Waring blendor in which 100 cc. of sterile physiological solution of sodium chloride has been placed previously. Blending is carried out for 4 minutes. The chamber of the Waring blendor was provided by the manufacturer, at our request, with an outlet near the bottom, in which a rubber vaccine bottle stopper is fitted. The chamber is connected with a Cornwall pipetting unit (Becton, Dickinson, and Co., No. 1251), by means of rubber tubing and a 20 gauge needle. This pipetting unit, which is equipped with a two way valve for continuous injection, is provided with a 21 gauge 2 inch needle and mounted on a metal stand at a convenient height. By means of specially constructed apparatus, injections are made with a foot treadle. The assembly of apparatus is shown in Text-fig. 1.

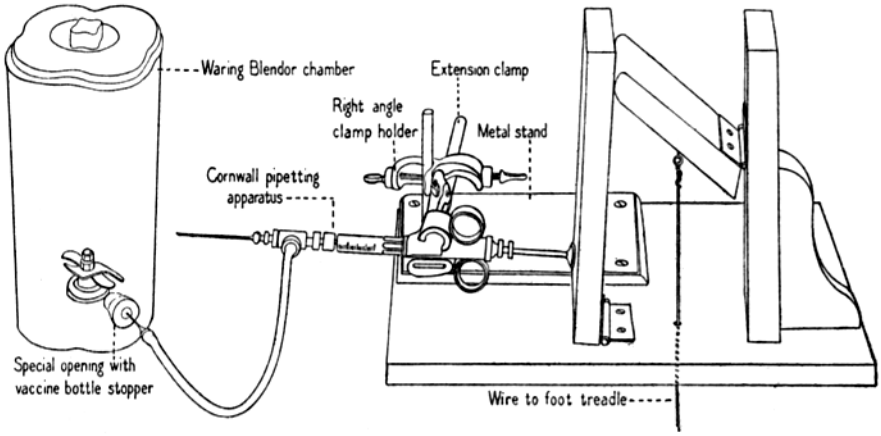
The fertile eggs to be injected, after preliminary incubation at 37.5° for 5 days, and after discarding those showing embryo death in the process of candling, are swabbed with iodine at the ends containing the air sacs. A minute hole is then punched in each (at the air sac end) by means of an automatically released blood lancet mounted on a metal stand. The point of the lancet is ground to a conical shape. With a little practice, by adjusting the depth of penetration, and by not holding the egg too firmly against the guard, small round holes may be punched with great speed, and without cracking the shell. The end of the shell is again swabbed with iodine after punching.

The actual injection of each egg is carried out by bringing the minute orifice in the shell in contact with the end of the fixed needle of the pipetting unit, pushing it in a straight line until the end of the needle in the yolk sac is approximately under the embryo, the position of which has been marked in lead pencil at the last candling, and injecting 0.1 cc. of the emulsion containing the rickettsiae. (The persence of many rickettsiae and the absence of bacteria has been ascertained previously by the examination of giemsa-stained film preparations.) After injection of the rickettsiae the orifice is sealed with a solution of parlodion in absolute acetone. The syringe refills automatically in preparation for the next egg.

By this method, 150 eggs can be injected in 30 minutes, and the entire process, including removal from the incubator, punching holes, injection, and replacing in the incubator, takes only about 2 hours. In order to eliminate the possibility of contamination, the needle of

the pipetting apparatus is heated to dull redness after the injection of each of 12 eggs, and then washed and cooled by passing 1 cc. of the inoculum through it. (This is caught in a beaker containing formalin, in order to protect the operator.)

Subsequent introduction of the agents to be tested has been carried out by again swabbing with iodine, injecting the solution, and resealing with parlodion. For the injection, 1 cc. tuberculin syringes provided with 24 gauge 1 inch needles are used. A separate syringe is used for each egg to avoid contamination. The solutions are made up separately and stored in sterile rubber-capped vaccine bottles. It has not been found necessary to work under a glass frame in order to avoid contamination.



TEXT-FIG. 1. Apparatus designed for rapid injection of inoculum into the yolk sacs of fertile eggs. Depressing the foot treadle injects the desired amount of inoculum (governed by the adjustment of the pipetting apparatus) after the egg is in position. When the foot treadle is released, the spring in the barrel of the syringe automatically refills the syringe and restores the levers to their original positions. The levers and the base of the main stand are constructed of soft pine. The main stand is clamped onto a laboratory table, through the top of which a hole is drilled to allow passage of the wire to the foot treadle.

Twelve to eighteen eggs served as controls for each experiment, while other groups of 8 to 12 eggs were injected with various concentrations of the substances to be tested, at varying intervals before or after the introduction of the rickettsiae. Estimates of the degree of infection were made from smear preparations of the yolk sac membrane of each egg, either after the death of the embryo or at other suitably chosen times. Such estimates were made independently by two different observers. In those rare instances where there was marked discrepancy in the results of the estimate, a careful restudy was carried out.

For testing chemotherapeutic effectiveness in laboratory animals, a method previously described, involving the use of mice of the dba strain (7, 8) was employed.

Penicillin and Para-Aminobenzoic Acid

A brief report on the rickettsiostatic action of penicillin in the yolk sac has been made previously (6), and a corresponding positive chemotherapeutic effect in typhus infection in mice has been observed (9) when large doses of penicillin were given early in the course of the disease.

Because of the synergistic effect of PABA on the bacteriostatic action of penicillin reported by Ungar (10), it seemed desirable to find out whether the rickettsiostatic activity of penicillin would be similarly enhanced by this agent. The following experiment was carried out for the purpose of answering this question.

Experiment 1.—The yolk sacs of 70 eggs were injected with typhus rickettsiae as described above. Fourteen eggs served as controls for the experiment as a whole. 48 hours after the injection of rickettsiae, 6.6 mg. of para-aminobenzoic acid dissolved in 0.4 cc. of distilled water was introduced into the yolk sacs of 8 eggs. Eight eggs received 1000 units of penicillin, 8 received 100 units, and 8 received 10 units. The penicillin was dissolved in distilled water and injected in three divided doses of 0.4 cc. each on the 2nd, 4th, and 6th days after injection with rickettsiae. Three additional groups of 8 eggs each were given penicillin as described above, and in addition to the penicillin each egg received 6.6 mg. of para-aminobenzoic acid in 0.4 cc. of distilled water 48 hours after injection with rickettsiae. Assuming uniform solution in the entire fertile egg, this would represent a concentration of para-aminobenzoic acid of about 1:6000. Actually the concentration was probably greater, since the compound is presumably not soluble in the fat globules of the yolk. Another unknown factor is the distribution of the compound between the cells and the extracellular fluid.

Results.—The results of this experiment are shown in Table I, in which each symbol represents an observation on an individual egg. It is seen that 1000 units of penicillin exerted a definite inhibitory action, confirming previous experiments (6). A slight inhibitory action by 100 units and 10 units of penicillin is suggested, but the differences between these two groups and the control group are probably not significant. Survival of embryos beyond the 12th day is seen only in eggs receiving 1000 units of penicillin alone, para-aminobenzoic acid alone, or smaller amounts of penicillin in combination with para-aminobenzoic acid. Para-aminobenzoic acid in the concentration used is seen to have an inhibitory action on rickettsial growth about equal to that of penicillin. This result was quite unexpected. The strongly rickettsiostatic action of para-aminobenzoic acid by itself made the experiment inadequate for its original purpose of discovering a possible synergistic effect of this substance with penicillin, although the complete absence of rickettsiae in the group of eggs treated with 1000 units of penicillin plus PABA may be significant.

Experiment 2.—This experiment was carried out for the purpose of determining the minimum concentration of para-aminobenzoic acid which would be effective, and also to compare the effect of neutralized para-aminobenzoic acid with the acid itself. Eighteen control eggs were injected with richettsiae in the usual way. Twelve eggs received 6.6 mg. of PABA dissolved in 0.4 cc. of distilled water, 8 eggs received 3.3 mg., 8 eggs 0.82 mg., and 8 eggs 0.20 mg. Twelve eggs received 6.6 mg. of PABA in a solution which had been made neutral to litmus by the addition of sodium hydroxide. All treatments were given 48 hours after the njection of rickettsiae.

Results.—The results of this experiment (Table II) indicate that 3.3 mg. of PABA had approximately the same rickettsiostatic activity as 6.6 mg., while

TABLE I

Growth of Typhus Rickettsiae in Eggs Treated with Combinations of Penicillin and PABA

Day following inoculation	Control	6.6 mg. PABA	1000 units penicillin	1000 units penicillin + 6.6 mg. PABA	100 units penicillin	100 units penicillin + 6.6 mg. PABA	10 units penicillin	10 units penicillin + 6.6 mg. PABA
	Degree of infection	Degree of infection	Degree of infection	Degree of infection	Degree of infection	Degree of infection	Degree of infection	Degree of infection
3		−	−	− −	−	(+)	−	− − −
4	−				−	−		− −
6	++							
7	++ +++	(+) −	−		+ ++	− −	++ +++	(+)
8	++++++ ++++ ++++++			−			++++	
9	++++++ ++++++ ++++ ++++	++			++++ ++++ +++++			++
10	+++++	++				(+)	++++ ++++	
11		−					++++	
12	+++ +++++	−*	(+)	−*	++++	(+)*	++++	−*
13		(+)*	− + −	−*		−*		
15			−* −*	− −* −*		−*		

− No *rickettsiae* recognizable with certainty.
(+) Less than one rickettsia per oil immersion field.
\+ 1–10 rickettsiae per oil immersion field.
++ 10–100 rickettsiae per oil immersion field.
+++ 100–1000 rickettsiae per oil immersion field.
++++ 1000–5000 rickettsiae per oil immersion field.
+++++ 5000–8000 rickettsiae per oil immersion field.
++++++ 8000–12,000 rickettsiae per oil immersion field.
* Embryo alive at time of examination.

TABLE II

Growth of Typhus Rickettsiae in Eggs Treated with Different Concentrations of PABA and with Neutralized PABA

Day following inoculation	Control	1/3000 (6.6 mg.) PABA	1/6000 (3.3 mg.) PABA	1/24,000 (0.82 mg.) PABA	1/96,000 (0.20 mg.) PABA	1/3000 (6.6 mg.) PABA (neutralized)
	Degree of infection	Degree of infection	Degree of infection	Degree of infection	Degree of infection	Degree of infection
4	++ ++ ++		(+) ++	(+)	(+)	(+)
5	+++ ++	++ ++ +++ +++ +++	++	+++++ +++ ++ ++	++	++ ++
6	++++ +++++ ++++ ++++ ++++ ++++ +++++ ++++	++++ ++ ++		++++	++++	+++
7	++++++ ++++		+	+++++ +++	++++++ +++++ +++++ +++++ ++++++	+++ + (+)
8	++++++ +++++ +++++	−*	+++*			(+) +
9		+* ++*	++			+++*
10		+++*				++*
11			++* +++			++*

For explanation of symbols, see Table I.

* Embryo alive at time of examination.

0.82 mg. and 0.20 mg. had no apparent effect. The action of neutralized PABA did not differ significantly from that of the acid itself. The inhibition of

rickettsial growth is less striking in this experiment than in experiment 1, probably because the organisms began to multiply earlier, and had attained a relatively higher concentration before treatment was begun. It is to be noted that survival of embryos beyond the 8th day occurred only in eggs receiving 3.3 mg. or more of PABA.

Experiment 3.—In this experiment, meta-aminobenzoic acid and ortho-aminobenzoic acid were compared with para-aminobenzoic acid from the point of view of rickettsiostatic activity. The chemicals to be tested were introduced into the yolk sacs 24 hours before the injection of rickettsiae.

Results.—Inhibition of rickettsial growth by para-aminobenzoic acid is seen (Table III) while the ortho and meta forms, in similar concentration, are without demonstrable action. Survival of embryos beyond the 7th day is noted only in eggs receiving para-aminobenzoic acid.

In another experiment, sodium benzoate, in a concentration of 6.6 mg. per egg, was found to have no demonstrable rickettsiostatic action.

Experiment 4.—This experiment was planned to test the therapeutic effectiveness of para-aminobenzoic acid in murine typhus infection in mice. The experimental conditions were identical with those used in previous work (9) in which the therapeutic action of penicillin was demonstrated. The usual death rate in the control group under these conditions was 100 per cent. Sixteen mice of the dba strain were injected intraperitoneally with 0.5 cc. each of a 10 per cent emulsion of brain tissue from a mouse dying of murine typhus after intraperitoneal injection of rickettsiae. These mice were kept at a room temperature ranging from 65–73° F. Eight of the mice were fed Purina dog chow in its original biscuit form. The other 8 mice were fed Purina dog chow, ground to a fine powder, with which 3 per cent of para-aminobenzoic acid was thoroughly mixed. All mice were given water *ad lib.* The treated mice were started on their diet containing PABA immediately after injection with rickettsiae.

Experiment 5.—This was a duplication of experiment 4, except that 30 mice were used, fifteen of which had PABA added to their food. One mouse in the PABA series was killed on the 6th day for information.

Results.—The mice apparently ate the powdered dog chow containing the PABA quite freely after the first 12 to 24 hours. One mouse in experiment 4 is believed to have died of starvation on the 4th day, however. The second mouse dying in experiment 4 on the 7th day probably died of typhus, although unfortunately smears were not made from the peritoneal cavity. This was the only mouse on the PABA diet which died from typhus infection presumably.

The results of experiments 4 and 5 are shown in Tables IV and V. A survival rate approaching 100 per cent is seen in the mice fed dog chow with PABA, as contrasted with a survival rate of zero in the mice fed dog chow alone.

Smears were made from the peritoneal cavities of all control mice shortly after death, and large numbers of rickettsiae were present in all instances. No evidence of secondary bacterial infection was found postmortem in any of the mice. A smear from the peritoneal cavity of the mouse in the PABA series,

TABLE III

Growth of Typhus Rickettsiae in Eggs Treated with Para-, Ortho-, and Meta-Aminobenzoic Acid

Day following inoculation	Control	6.6 mg. PABA	6.6 mg. MABA	6.6 mg. OABA
	Degree of infection	Degree of infection	Degree of infection	Degree of infection
2		−	−	−
		−	−	+
4	++	+	+	
	−	−	+	++
	+++	−		++
	+			+++
	++			
	+			
	++			
	++			
	+			
	++			
5	+++	−	++	+++
	++		++++	+++++
			+	+++
			+++	++++
6	++++++	+	++++++	++++++
	++++++	++	+++++	++++++
	++++++	++	++++	++++++
		−*	++++++	
			++	
			++	
7	++++++	(+)*	++++++	++++++
	++++	+*	++++++	++++++
	++++++		++++++	+++++
	++++		++++++	
8		−*		
10		−*		
		−*		
		(+)*		
		(+)*		

For explanation of symbols, see Table I.

* Embryo alive at time of examination.

killed on the 6th day with no sign of illness, showed rare extracellular rickettsiae, and an occasional cell containing 15 to 20 organisms.

Sodium Fluoride

Sodium fluoride was chosen as a test substance because of its well known effect of inhibiting glycolysis by interfering with phosphorylation. Preliminary experiments showed that the injection of 5 mg. of this substance into the yolk sac caused death of the embryos in 12 to 24 hours, while injections of 1 mg. or less were well tolerated.

In two experiments which will not be published in detail, series of eggs treated with 1 mg. and 0.1 mg. of sodium fluoride developed heavy rickettsial infection 24 hours earlier than the controls, and embryonic death occurred sooner than

TABLE IV

Therapeutic Effect of PABA in Food on Murine Typhus Infection in Mice

Mice injected	Diet	Illness	Death	Time of death	Survived
8	Dog chow plus PABA	3	2	One on 4th day One on 7th day	6
8	Dog chow alone	8	8	All on 7th day	0

TABLE V

Therapeutic Effect of PABA in Food on Murine Typhus Infection in Mice

Mice injected	Diet	Illness	Death	Time of death	Survived
15	Dog chow plus PABA	0	One killed for study		14
15	Dog chow alone	15	15	6th and 7th days	0

in the controls. In a repetition of this experiment, however, using 1 mg. of the substance, no significant differences were noted between the control and the treated groups.

Experiment 6.—Sixteen eggs were injected with rickettsiae in the usual way. Forty-eight hours later 0.5 mg. of sodium fluoride in 0.5 cc. of distilled water was introduced into the yolk sacs of 8 of these eggs.

Results.—Only one of the control eggs showed an occasional rickettsia, and guinea pig inoculation from the yolk sac of this egg gave entirely negative results. Seven of the 8 eggs receiving sodium fluoride developed visible rickettsiae, and in several eggs rickettsiae were numerous. The results are seen in

Table VI. A guinea pig inoculated from one of the heavily infected eggs developed typical murine typhus after an incubation period of 3 days.

The failure of the control eggs to develop the usual picture of heavy infection is a phenomenon which has occurred in about one-fourth of our experiments. Whether it is due to insusceptibility of the eggs to infection, or to injection of material containing non-viable rickettsiae is not clear. The sodium fluoride injection, however, apparently produced conditions favorable for rickettsial growth in this experiment.

TABLE VI

Effect of Sodium Fluoride on Rickettsial Multiplication in the Yolk Sac

Day following inoculation	Control	0.5 mg. sodium fluoride
	Degree of infection	Degree of infection
3		−
4	−	+
7		+
8	−*	+++
9		++
11	−*	++*
12	(+)*	++++*
13	−*	+*
14	−*	
	−*	
	−*	

For explanation of symbols, see Table I.

* Embryo alive at time of examination.

Penicillin and Para-Aminobenzoic Acid with Sodium Fluoride

Experiment 7.—The purpose of this experiment was to determine whether or not sodium fluoride would interfere with the rickettsiostatic action of penicillin and para-aminobenzoic acid. Forty-two eggs were injected with richettsiae in the usual way. Forty eight hours later, 12 eggs received injections of 6.6 mg. of para-aminobenzoic acid and 0.1 mg. of sodium fluoride. Similarly, 12 eggs received injections of 666 units of penicillin and 0.1 mg. of sodium fluoride.

Results.—From the results as shown in Table VII it is clear that the suppression of rickettsial growth by PABA and by penicillin was not markedly influenced by sodium fluoride in the concentration used.

Negative Results

Using the technic described above, the following agents have been tested for their ability to alter the multiplication of murine typhus rickettsiae in the yolk sac, when introduced in single doses 24 to 48 hours after the injection of

rickettsiae. The figures following each agent indicate the amount injected into each egg. Riboflavin, 6 and 15 mg.; thiamin chloride, 3 and 15 mg.,

TABLE VII

Effect of Sodium Fluoride in Combination with Para-Aminobenzoic Acid and Penicillin

Day following inoculation	Control	6.6 mg. PABA and 0.1 mg. NaF	666 units penicillin and 0.1 mg. NaF
	Degree of infection	Degree of infection	Degree of infection
4	++	(+)	−
	++	(+)	(+)
	++	−	(+)
		−	
		+	
5	+++	++	(+)
	++	++	(+)
		−	(+)
			++
6	++++		++
	+++++		++
	++++		++
	++++		
	++++		
	++++		
	+++++		
	++++		
7	++++++	−	++
	++++		
8	++++++	(+)*	(+)
	+++++		
	+++++		
9		++++	
11		++	

For explanation of symbols, see Table I.

* Embryo alive at time of examination.

choline chloride, 4 and 24 mg.; Bc, 0.0375 gamma; biotin, 0.1 mg.; physostigmine, 0.05 mg.; iodoacetic acid, 0.1 mg.

DISCUSSION

Agents injected into the yolk sac in the above experiments were in intimate contact with the entodermal cells in which the rickettsiae grow. The observed

inhibition or stimulation of rickettsial growth may have been brought about (1) by direct action on the enzymes concerned with the metabolism of the rickettsiae themselves or (2) by modifying the metabolism of the entodermal cells in such a way as to upset the delicate state of symbiotic equilibrium which exists between the rickettsiae and their host cells.

It has not been possible in any instance to determine with certainty which of these mechanisms is involved. In the case of penicillin, direct action on the metabolic enzymes of the rickettsiae themselves seems likely, since this strongly bacteriostatic substance has not been shown to modify the metabolism of animal tissues, and is probably an enzyme inhibitor rather than an enzyme activator. PABA, on the other hand, is believed to be a member of the B group of vitamins, and its effect may, like that of riboflavin (3), be due to stimulation of the metabolism of the host cells.

The increased survival rate in typhus-infected mice fed toluidine blue, reported by Peterson (11), is of particular interest in this connection. This dye has been shown (12) to increase the oxygen uptake of tissues, an effect which is neutralized by KCN. Assuming that the action of toluidine blue in mice is the result of this increased oxygen uptake, Peterson's work may be taken as confirmatory evidence that a high metabolic activity is unfavorable for the multiplication of rickettsiae in cells.

The resistance of cells to rickettsial infection may depend on a variety of factors which include (1) temperature (8), (2) presence in the cells of intact mechanisms for their own respiration and probably for other metabolic processes, (3) absence from the cells of metabolic enzymes essential for rickettsial growth, and (4) presence in the cells of enzyme systems antagonistic to rickettsial growth. Factors 3 and 4 may determine absolute "natural" immunity, while variations in factors 1 and 2 may determine the severity of infection in the case of naturally susceptible cells. The peritoneal lining cells of rats may be made abnormally susceptible to rickettsial infection by partial riboflavin deficiency (3), and the administration of riboflavin has an immediate and striking rickettsiostatic action under these conditions (13). In normal mice the administration of large amounts of riboflavin is therapeutically ineffective (14).

On the basis of this hypothesis, an agent which is therapeutically effective in typhus infection in animals of one species might be ineffective in animals of another species, because of existing differences in metabolism. Murine typhus rickettsiae multiply in the peritoneal cavity of the normal rat, but produce little or no obvious evidence of illness. In the normal mouse, under the conditions used in the above experiments, murine typhus is a fatal disease. The addition of para-aminobenzoic acid to the food makes the disease in mice inapparent, as it is in the normal rat. It is to be noted that a few rickettsiae were

found in the peritoneal cells of the mouse of the PABA series which was killed for information.

The stimulating effect of sodium fluoride, under certain conditions, on rickettsial growth in the yolk sac may be due to its interference with carbohydrate metabolism. By the use of this agent or of other similar agents, it may be possible to render the entodermal cells of the yolk sac susceptible to infection by rickettsia-like organisms to which they are normally resistant.

The extension of the type of investigation described above to the study of the metabolism of viruses seems to offer interesting possibilities for future exploitation.

SUMMARY

By injection into typhus-infected yolk sacs, a number of agents were tested for possible inhibition or acceleration of rickettsial growth. The previously reported rickettsiostatic activity of penicillin was further confirmed.

Para-aminobenzoic acid, in single injections of 6.6 mg. and 3.3 mg. giving initial concentrations of approximately 1:6000 and 1:12,000 was found to have rickettsiostatic activity approximately equal to that of penicillin. No conclusion could be drawn regarding the possibility of a synergistic action of para-aminobenzoic acid and penicillin. Para-aminobenzoic acid neutralized with sodium hydroxide was found to be as effective as the acid itself, when given in single injections of 6.6 mg. Sodium benzoate, as well as the ortho and meta forms of aminobenzoic acid were found to be ineffective when given in similar amounts.

Para-aminobenzoic acid, when added to the food in a concentration of 3 per cent, was shown to have a remarkably effective chemotherapeutic action on murine typhus infection in mice.

Sodium fluoride was found at times to accelerate the growth of rickettsiae in the yolk sac, and to cause heavy infection under conditions such that the controls showed practically no multiplication of the organism. When rickettsiostatic substances (penicillin and para-aminobenzoic acid) were combined with sodium fluoride, their rickettsiostatic activity was not demonstrably changed. Other agents studied and found not to affect rickettsial multiplication are listed. The possible mechanisms involved in the observed inhibition and stimulation of rickettsial growth under these conditions are discussed.

Note: As this paper was going to press, it was learned that favorable therapeutic effects from para-aminobenzoic acid in experimental typhus infection had been described previously in unpublished confidential reports in the files of the United States of America Typhus Commission. The authors therefore wish to disclaim priority in the above observations, insofar as they deal with this chemical substance. Our observations were made independently, and without knowledge of this unpublished work.

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