sulting from the Cu catalyzed oxidation of 0.05 M ascorbic acid.

DISCUSSION

The role of Cu in the virucidal action of ascorbic acid is still not clearly defined, though our preliminary observations have indicated that KCN inhibits the virucidal action of ascorbic acid presumably by combining with Cu to form a stable compound.

It is of interest to note that the bactericidal activity of Penicillin B has also been reported as being due to the production of H_2O_2. The Penicillin B was found to be a glucose oxidase producing H_2O_2 in the presence of glucose and oxygen.[9]

SUMMARY

The theoretical yield of H_2O_2 formed during the oxidation of a virucidal solution of ascorbic acid approximates the virucidal action of an equivalent amount of H_2O_2. Both the action of ascorbic acid and H_2O_2 are completely neutralized by catalase. The action of ascorbic acid against influence A virus may therefore be explained as being due to the H_2O_2 formed during the Cu catalyzed oxidation of ascorbic acid.

The observed *in vitro* virucidal activity of ascorbic acid obviously can not be utilized therapeutically because of the presence of catalase in body tissues.

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BLOOD, URINE AND FECAL LEVELS OF STREPTOMYCIN IN THE TREATMENT OF HUMAN INFECTIONS OF E. TYPHOSA[1,2,3]

THE clinical studies of the effect of streptomycin on the course of typhoid infections in humans will be reported by Reimann, Elias and Price.[4]

The potency assay of streptomycin depends on a cylinder-plate technique, similar to the method used in penicillin assay, in which a strain of *B. subtilis* was substituted for *S. aureus*.[5] This procedure is also like that used in the assay of streptothricin.[6]

During this investigation, patients were given streptomycin in dosages ranging from one million to four million units daily. The drug was given by the intramuscular or intravenous routes and, in one patient, by the oral route which, on discontinuance, was followed by injection, using the intravenous drip method. Intramuscular and oral doses were given at three-hour intervals.

Blood and serum samples were usually collected after the first dose at intervals of 1, 2, 3, 6, 12, 24, 36, 48, etc., hours of treatment. Samples were also drawn at short intervals on discontinuance of dosage. Urine samples were collected at a number of short intervals following the initial dose and again on discontinuance of dosage. In the interim, 24-hour urine collections were made to determine the amounts of streptomycin present. In two cases fecal samples were assayed, when possible, on the basis of 24-hour collections.

INTRAMUSCULAR AND INTRAVENOUS DOSAGE

These routes of administration produced similar blood and serum levels in direct proportion to dosage. Four million units daily, given intravenously, resulted in whole blood levels with a mean of 12 units per ml and a mean of 28 units per ml of serum. Peak levels were reached within a few hours after the start and disappeared within 24 hours after cessation of dosage.

With an intramuscular administration of 20 million units over six days, 44 per cent. of the streptomycin was recovered in the urine. Following the intravenous dosage of 28 million units over 7 days, 70 per cent. of the streptomycin was accounted for in the urine. Urinary excretion appeared within 1½ hours, with only traces remaining 72 hours after cessation of dosage.

Fecal levels were not determined with intramuscular dosage, nor were total recoveries determined by intravenous administration. However, four million units daily, by the intravenous route, produced from 100 to 130 units per gram of feces.

ORAL DOSAGE FOLLOWED BY INTRAVENOUS ADMINISTRATION

Four million units daily, by oral ingestion, produced no demonstrable blood level. Approximately 1 per cent. appeared in the urine, while at least 64 per cent. was eliminated in the stools over the period of oral dosage, with as high as 21,700 units per gram of fresh feces.

When oral administration was suddenly changed to intravenous injection, streptomycin promptly appeared in the blood and serum at levels comparable

[9] O. Schales, *Arch. Biochem.*, 2: 487, 1943.

[1] The method for the assay of streptomycin in citrated blood, hemolysed with saponin, and urine was obtained through the courtesy of Dr. J. M. Carlisle and Dr. D. F. Robertson, of the Merck Institute for Therapeutic Research, Rahway, N. J.

[2] This work was conducted in the laboratory of Dr. H. A. Reimann in Jefferson Medical College, Philadelphia, Pa.

[3] Detailed methods of procedure will be presented in a subsequent publication.

[4] H. A. Reimann, W. F. Elias and A. H. Price, *Jour. Am. Med. Asn.*, in press.

[5] H. J. Robinson and D. G. Smith, *Jour. Pharmacol. and Exp. Therap.*, in press.

[6] J. W. Foster and H. B. Woodruff, *Jour. Bact.*, 45: 408, 1943.

to those observed previously, 65 per cent. was excreted in the urine and only 2 per cent. in the feces.

Table I presents representative data of streptomycin recoveries after various methods of administration.

The general impression prevailed throughout these tests that streptomycin in water solution, and in its passage through the body, was extremely stable, and it is very probable that recoveries in the urine and feces were actually greater than reported here. In this connection it is certain that total daily yields of urine and feces were not obtained because of uncontrolled eliminations by these very ill patients.

Resistance Tests

Robinson, Smith and Graessle,[7] employing the agar plate technique, reported that the growth of *Eberthella typhosa* was inhibited by one unit of streptomycin.

The streptomycin resistance of the typhoid organism, isolated from blood and stool cultures during this investigation, was measured by a broth culture technique in which 5 ml volumes of broth contained 1,000 organisms per ml, and concentrations of streptomycin ranging from 0.5 unit to 20 units per ml. Readings were made 24 and 96 hours after incubation at 37° C.

Three cultures from three patients, including two from stools and one from blood, isolated prior to streptomycin therapy, were all killed by 6 units, but not by 4 units, of streptomycin per ml of broth.

A culture, isolated from blood after nine days' intramuscular dosage of one million units/24 hours, showed no change in resistance.

A stool culture, isolated after a total oral dosage of 15 million units and a subsequent 8 million units administered intravenously, likewise demonstrated no change in resistance.

Modification of the procedure, in which Widal negative human serum was added to the broth to a concentration of 10 per cent., did not materially change the activity of streptomycin to all the above cultures.

A control, consisting of a stock culture of *Eberthella typhosa* maintained for several years on agar culture, was killed by 2 units, but not by 1 unit, of streptomycin in broth and serum-broth.

In connection with the similarity in resistance among the cultures isolated during this investigation, it is of considerable interest that positive isolations were made in two persons whose stools contained 40 units per gram and 145 units per ml. Bearing in mind the enormous dosages administered, in contrast to the *in vitro* resistance, it is possible that some inhibitory substance to streptomycin exists in the human body. Because of the considerable recoveries, streptomycin was not destroyed by this agent, but its activity was very much reduced.

TABLE 1

Streptomycin Levels in Blood, Serum, Urine and Feces After Various Routes of Administration

Patient	Interval dosage in units	Route	Blood† units/ml	Serum† units/ml	Urine levels				Fecal level*			
					Volume	Units/ml	Interval recovery in units	Per cent. recovery based on interval dosage	Fresh weight	Units/gram	Interval recovery	Per cent. recovery based on interval dosage
								per cent.				*per cent.*
H	2 million	intramuscular	5 to 7	13 to 14	2,580 ml	225	581,000	29	....	....	....	....
	4 "	"		15 to 20	2,200	640	1.4 million	32	....	....	....	....
M	4 million	intravenous	12 (Mean)	27 (Mean)	3,100	1,000	3.1 million	77	....	....	....	....
	2 "	"	5 to 6	13 to 14	2,920	470	1.37 "	68	....	....	....	....
	24 hours after cessation of dosage				500	290	145,000		58.3 grams	40	2,330	
F	1 million	oral	none	none	2,200	4	8,800	0.9	181 grams	4,000	724,000	72
	2 "	"	"	"	1,900	12	22,800	1.1	732 grams (enema)	3,000	2.2 million	110
	4 "	"	"	"	1,300	29	38,000	0.95	113 grams	8,400	923,000	23
	2 "	intravenous	6	19	3,200	330	1.06 million	53	180 ml (enema)	145 u/ml	26,100	0.7
	4 "	"	15	32	3,500	740	2.6 "	65	231 grams	220	51,000	1.3

* Fecal recoveries varied considerably from day to day, but over-all recoveries amounted to at least 64 per cent. by oral dosage and approximately 1 to 2 per cent. by intravenous administration.

† These values are representative of the blood and serum levels existing at 12-hour intervals after initiation of dosage.

Conclusions

(1) Streptomycin levels in blood, serum, urine and feces were easily determined.

(2) Intramuscular and intravenous dosages were comparable with respect to demonstrable blood levels, high urine recovery and, in intravenous dosage, a low fecal recovery.

(3) Oral administration produced no demonstrable blood level, a very low urinary recovery, but an extremely high fecal content. This is indicative that

[7] H. J. Robinson, D. G. Smith and O. E. Graessle, *Proc. Soc. Exp. Biol. and Med.*, 57: 226, 1944.

streptomycin, under the conditions of these investigations, was not readily absorbed through the intestines.

(4) The *in vitro* streptomycin resistance of cultures, isolated during therapy, did not differ from cultures isolated from blood and stools prior to the administration of streptomycin.

(5) The contrast between the very large dosages coupled with blood and fecal isolations during therapy, and the comparatively low *in vitro* resistance of these cultures, indicated the possible presence in the body of a substance inhibitory to streptomycin.

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PARA-AMINOBENZOIC ACID—ITS EFFECTIVENESS IN SPOTTED FEVER IN GUINEA PIGS[1]

IN contrast with the spectacular successes achieved by chemotherapy in bacterial infections, chemotherapy in viral and rickettsial diseases is in general noneffective. This applies particularly to the sulfonamide agents. Greiff and Pinkerton[2] have shown that sulfanilamide does not inhibit rickettsial growth in egg yolk-sac. Still more striking are the observations by Snyder, Maier and Anderson[3] that the sulfonamide drugs have a deleterious effect in experimental typhus. These phenomena, which offer another evidence of the apparently different metabolic requirements and activities of the intracellular rickettsiae, led Greiff, Pinkerton and Moragues[4] to the use of "enzyme activators" in rickettsial infections. The inhibitory action of p-aminobenzoic acid (PABA) on rickettsial growth was observed in egg yolk-sacs infected with murine typhus rickettsiae. A favorable effect of this drug on murine typhus infection in mice was also reported.[4] The beneficial effect of PABA on the clinical course of louse-borne typhus in man was established by the United States of America Typhus Commission unit in Egypt.[5]

On the basis of these observations PABA was tested by us in guinea pigs infected with Rocky Mountain spotted fever. The strain of tick origin (Rocky Mountain Laboratory) proved highly virulent to guinea pigs: the regular 72 hours' incubation period is followed invariably by high fever of 5 to 8 days' duration. The death-rate in infected guinea pigs is 100 per cent.

The present work was directed into the possible protection of animals against the disease by the use of PABA. For this purpose guinea pigs were given the drug in proportion of 2 gm of the powder per each 100 gm of high protein feed *ad lib.* The administration of this mixture was started shortly after the intra-abdominal injection of the infective material (spleen or blood). In other series the drug was given 24 hours prior to the infection or 24, 48 and 72 hours thereafter. The daily PABA treatment was then continued for 7 to 10 days, after which time it was withdrawn.

As compared with the clear-cut febrile and fatal disease in control animals a striking change in the response of test guinea pigs was observed. Out of 12 guinea pigs in which PABA intake preceded the injection by 24 hours three animals showed fevers of 1 to 3 days' duration; two guinea pigs reacted with slight and late elevations for 1–2 days, while seven animals remained completely afebrile. Two deaths were recorded. In another series of 17 guinea pigs treated with PABA beginning with the day of spotted fever injection, eleven animals remained afebrile during two weeks' observation, three reacted with one or two days' fever, two developed typical spotted fever and died, while one died showing no fever. As a whole, in 80 per cent. of test guinea pigs the fever was either entirely absent or reduced to abortive mild attacks.

When PABA was administered during the incubation period 30 per cent. of guinea pigs remained afebrile even when the drug was given 72 hours after injection. However, the animals of this series showed a rather erratic appetite, resulting in an inefficient intake of PABA.

These facts indicate that PABA, given before or shortly after infection, can prevent the appearance of clinical manifestations of the fatal spotted fever. In the large majority of test animals the visible symptoms (fever and scrotal reaction) were entirely absent. Nevertheless, some afebrile guinea pigs showed pathological lesions (splenomegaly, pneumonitis) typical for spotted fever. When the spleen was injected into normal guinea pigs the latter reacted with typical spotted fever like the controls. The surviving afebrile animals were then reinoculated with spotted fever rickettsiae and showed complete immunity. It seems, therefore, that the absence or mildness of clinical symptoms indicate rather a suppressive than destructive effect of p-aminobenzoic acid on spotted fever rickettsaie with the result of

[1] Study assisted by a grant from the American Foundation of Tropical Medicine, Incorporated.

[2] D. Greiff and H. Pinkerton, *Proc. Soc. Exp. Biol. and Med.*, 55: 116–119, 1944.

[3] J. C. Snyder, J. Maier and C. R. Anderson, Report to the Division of Med. Sciences, Nat. Res. Counc., December 26, 1942.

[4] D. Greiff, H. Pinkerton and V. Moragues, *Jour. Exp. Med.*, 80: 561–574, 1944.

[5] A. Yeomans, J. C. Snyder, E. S. Murray, C. J. D. Zarafonetis and R. S. Ecke, *Jour. Am. Med. Asn.*, 126: 349–356, 1944.