BIOTIN-BINDING BY PARENTERALLY-ADMINISTERED STREPTAVIDIN OR AVIDIN

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The nutritional deficiency originally called "egg white injury" (Boaz, 1924) was shown to be reversed by biotin (Gyorgy, 1939, and Gyorgy et al., 1940) and caused by feeding a biotin-binding protein named avidin. Originally isolated from the whites of hens eggs (Eakin et al., 1940, 1941), avidin has been reported to occur only in the white of eggs or the egg jelly of birds and amphibians (Hertz and Sebrell, 1942), the oviduct tissue of laying birds (Fraps et al., 1943) and in small amounts in certain sperm cell preparations (Jones and Briggs, 1962). When avidin or raw egg white is fed to rats, the avidin binds biotin in the intestine, presumably forming a non-absorbable complex, and signs of biotin deficiency develop in the animal. One report of the parenteral use of avidin was made by Gyorgy and Rose (1941). This route not only did not result in the typical "toxic effect" associated with enteral avidin, but even seemed to have a therapeutic effect for animals already suffering from biotin deficiency brought on by an avidin-containing diet. These workers pointed out, however, that the probable contamination of their avidin preparation with biotin made the interpretation of their experiments equivocal.

Streptavidin is the first avidin-like material ever shown to be elaborated by a microorganism. It is described by Tausig and Wolf, whose studies demonstrated that the biotin-binding ability of streptavidin is analogous to that of avidin (Tausig and Wolf, 1963). The same Streptomycetes that produce streptavidin also produce a biotin-reversed antibiotic agent

currently designated only as "S" (Chaiet et al., 1963). In a synthetic medium this antibiotic has gram-negative antibacterial activity reversed by biotin. "S" is inactive in a vitamin-rich medium unless streptavidin or avidin also is present. Under these conditions, the resulting inhibition of bacterial growth also can be reversed by biotin (Tausig and Wolf, 1963). Because of this latter observation, the ability of streptavidin or avidin to be given parenterally as an adjunct to the antibacterial agent "S" was investigated. These studies are reported here.

Current Investigations

Female mice of the Sharp and Dohme strain, weighing 23-25 g, are infected intraperitoneally with approximately 50 cells of Salmonella schottmuelleri M.I., representing 5-10 lethal doses of this organism. The resulting infection in untreated mice is rapidly fatal, with death occurring in 36-48 hours. Neither "S" alone, streptavidin alone nor avidin alone protect the infected animals. Mice can be protected, however, by treating them with intraperitoneal injections of "S" and streptavidin or of "S" and avidin.[1] Table I shows the results of a typical experiment demonstrating these points.

TABLE I

THERAPEUTIC INTERDEPENDENCE OF "S" AND STREPTAVIDIN OR AVIDIN

Therapy, γ/dose			
S	Streptavidin	Avidin	S/T*
120	0	0	0/5
0	300	0	0/5
0	0	900	0/5
120	300	0	5/5
120	0	900	5/5

*Survivors over total number mice. Therapy intraperitoneally at 0, 6 and 24 hours after intraperitoneal infection with Salmonella schottmuelleri.

[1] Nutritional Biochemical Corporation, 2500 units/gram.

The amount of streptavidin or of avidin that has to be used with 120γ "S" in order to protect 50% (ED_{50}) of the infected mice was calculated from the data shown in Table II. For streptavidin the ED_{50} is 28γ plus 120γ "S", for avidin 84γ plus 120γ "S". With these preparations, therefore, avidin is one-third as active as is streptavidin.

TABLE II

TITRATION OF THE AMOUNT OF STREPTAVIDIN OR OF AVIDIN REQUIRED WITH 120γ "S" TO PROTECT MICE AGAINST INFECTION WITH SALMONELLA SCHOTTMUELLERI

γ/dose			γ/dose		
S	Streptavidin	S/T*	S	Avidin	S/T
120	0	0/5	120	0	0/5
120	18.7	1/5	120	56	1/5
120	75	5/5	120	225	5/5
120	300	5/5	120	900	5/5
0	300	0/5	0	900	0/5

*Survivors over total mice treated. Therapy intraperitoneally at 0, 6 and 24 hours after intraperitoneal infection with Salmonella schottmuelleri.

The ability of "S" + streptavidin or of "S" + avidin to protect mice from infection with Salmonella schottmuelleri can be reversed by the parenteral injection of biotin. To demonstrate this point groups of infected mice were treated intraperitoneally at the usual times with a mixture of 120γ "S" and 300γ streptavidin or with 120γ "S" and 900γ avidin. In addition, these mice were given, also intraperitoneally but at a different site from the antibiotic-adjunct mixture, either saline, 0.1γ, 1.0γ or 10γ biotin per dose. It can be seen from the data listed in Table III that mice given "S" + streptavidin or "S" + avidin mixtures were completely protected when they received in addition saline or 0.1γ biotin, but that

TABLE III

REVERSAL OF THERAPEUTIC ACTIVITY BY PARENTERAL BIOTIN

Biotin, γ/dose	Survivors over total mice* "S" + Streptavidin 120γ + 300γ	"S" + Avidin 120γ + 900γ
0	5/5	5/5
0.1	5/5	5/5
1.0	3/5	4/5
10	0/5	0/5

*All injections given intraperitoneally 0, 6 and 24 hours after intraperitoneal infection with Salmonella schottmuelleri.

this protection was partially reversed by 1.0γ and completely reversed by 10γ biotin.

Discussion

To our knowledge, this is the first report of the use of avidin or an avidin-like material as an adjunct to therapeutic activity. Although no assays of the biotin content of the tissues of our test animals were made, the data presented here suggest that the therapeutic activity of antibiotic "S" is dependent on the ability of streptavidin or of avidin to bind parenteral biotin. The graded reversal of such therapeutic activity by increasing amounts of biotin supports this view. Such parenteral binding would have to occur rapidly, because of the speed of the infection used. External signs of biotin deficiency were not observed on the animals, nor would they be expected since only three injections of avidin or streptavidin were given, and surviving mice were discarded one week after infection. In feeding experiments, signs of biotin deficiency are reported only after three to nine weeks on the egg white or avidin-containing diet (Emerson and Keresztesy, 1942).

Gyorgy and Rose (1941) have suggested that in a parenteral medium the avidin-biotin complex is split to release therapeutically active biotin. Although our data appear not to support this proposal, the great differences in the amount of avidin used and in the type and time-span of the two sets of experiments make discussion of this point difficult. In the light of the known in vitro interrelationships of "S", streptavidin or avidin, and biotin, and our demonstration of the biotin reversal of therapy, we interpret our result to signify that parenterally-administered streptavidin or avidin binds tissue biotin. These present data seem to be the first published ones indicating the parenteral binding of biotin by avidin or an avidin-like material.

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