(+++) is taken as the endpoint. The activity then increases, for example, from 20,500 to 10,500,000, that is over 500 times.

In Table 2 the influence of increasing biotin concentration on the lysis of acetone dried *M. lysodeikticus* is shown.

TABLE 2

Biotin concentration in micrograms	Lysozyme units per mg
0	640
0.01	640
0.1	2,600
1.0	5,000
2.0	5,000
4.0	10,000
6.0	41,000
8.0	82,000
10.0	164,000

The data reported here can not be explained with certainty at the present time. In analogy with many other enzyme systems, biotin might be considered as the prosthetic group of a protein carrier. This protein carrier would bind biotin, while the biotin-avidin complex would have lysozyme activity. In accordance with this hypothesis is the fact that avidin contains both free avidin and an avidin-biotin complex.[13]

However, all attempts to dissociate lysozyme into carrier and prosthetic group have failed so far. These attempts included dialysis in acid and alkaline solutions and electrophoresis. The preparation migrated cathodically at pH 7.80 with a sharp boundary ($u = +6.75 \times 10^{-5}$, Dr. D. Moore). It had an activity greater than 1,000 units per mg. The increasing activation of lysozyme with increasing biotin concentration may contraindicate a simple coenzyme effect of biotin, since the concentration of biotin is far greater than that of lysozyme. The biotin effect, however, apparently is not due to action on the test organisms, since it varies in extent with different preparations of lysozyme.

Aside from any hypothesis, however, the experiments reported in this paper definitely link biotin with lysozyme, a mucolytic enzyme concerned with defense against bacterial invasion. It remains to be seen whether a similar relationship holds true for other enzymes of this important group. It might be pointed out further that an enzyme with the bacteriological specificity of eggwhite lysozyme occurs in many if not in all lysozyme susceptible organisms.[5,12] Similar enzymes with other specificities have been demonstrated in many microorganisms. Such enzymes, which in high concentration partly or completely lyse the organisms from which they are derived, are probably concerned with bacterial multiplication.

KARL MEYER

[13] P. György and C. S. Rose, SCIENCE, 94: 261, 1941.

ON THE POSSIBLE IDENTITY OF "AVIDIN"[1] AND EGGWHITE LYSOZYME

COMPARATIVE studies on "avidin"[2,3] and eggwhite lysozyme[4] bring to light a number of common physical and chemical characteristics. Both seem to be present in the same fraction of raw eggwhite in the same relative abundance. Both have been concentrated by similar chemical procedures. The more similar procedures, *i.e.*, those of Meyer *et al.* for eggwhite lysozyme[4] and of Woolley and Longsworth for "avidin,"[3] have also yielded concentrates of qualitatively similar chemical elements, while the quantitative differences could be accounted for by the seeming differences in the degree of purification. Furthermore, the concept of "avidin" as "antibiotin" makes it difficult to reconcile the fact that whereas biotin is found in so many divergent organisms and tissues, "avidin" has hitherto been found only in whites of eggs or in the oviduct of certain species of frogs and fowl.[5] Moreover, the history of biotin, which was found to be identical with coenzyme R and vitamin H, further suggested that "avidin" may also be a more widely distributed substance.

These considerations led to a series of experimental procedures in which (A) a sample of known eggwhite lysozyme, prepared by Dr. Karl Meyer in October, 1937,[6] was subjected to the standard yeast test[7] for "avidin" activity, and (B) samples of "avidin" of known varying potencies,[8] prepared by Hoffmann-La Roche, Inc., were tested for lysozyme activity.[9]

All tests for both (A) and (B) proved strongly positive, and, furthermore, showed that the "avidin" activity in each "avidin" concentrate closely paralleled its lysozyme activity. The results in the (A) series of tests are shown in Table 1.

[1] The term "avidin" as used here refers to concentrates containing both "free avidin" and avidin-biotin complex.

[2] R. E. Eakin, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 140: 535, 1941.

[3] D. W. Woolley and L. G. Longsworth, *Jour. Biol. Chem.*, 142: 285, 1942.

[4] K. Meyer, R. Thompson, J. W. Palmer, and D. Khorazo, SCIENCE, 79: 61, 1934; *Jour. Biol. Chem.*, 113: 303, 1936; *ibid.*, 113: 479, 1936.

[5] R. Hertz and W. H. Sebrell, SCIENCE, 96: 257, 1942.

[6] The author is deeply indebted to Dr. Meyer for supplying him with this sample.

[7] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[8] Supplied through the courtesy of Dr. H. M. Wuest, of Hoffmann-La Roche, Inc., and Dr. Ira I. Kaplan, of Bellevue Hospital, New York City. The initial sample tested had a potency of 2,000 units per gm.

[9] The author hereby wishes to acknowledge his debt to Dr. Gustav J. Martin, of the Warner Institute for Therapeutic Research, New York City, for his invaluable assistance in carrying out the experimental work on the "avidin" activity of lysozyme, and to Dr. Meyer, who carried out the tests on the lysozyme activity of "avidin." Dr. Meyer will present his data on these tests, as well as on further tests initiated by himself, in a separate communication.

TABLE 1

"AVIDIN" ACTIVITY OF EGGWHITE LYSOZYME AS COMPARED WITH AN "AVIDIN" CONCENTRATE OF 50 UNITS PER GRAM (S.M.A.)

Tube No.	Biotin (milli-gammas)	S.M.A. Avidin concen. 50 units per g	Lysozyme	Control	Galvanometer reading density
1	50	..	..	√	100
2	50	..	..	√	98
3	50	1 mg	..	..	87.5
4	50	5 mg	..	..	26.00
5	50	..	1 mg	..	56.0
6	50	..	2.5 mg	..	17.5

The data thus show an "avidin" activity of about 100 units per gram for the lysozyme sample. Notice must also be taken of the fact that this sample had been kept for nearly six and a half years at room temperature, which makes it probable that it had lost some of its "avidin" activity, since György *et al.*[10] have observed that whereas the avidin-biotin complex resists the action of digestive enzymes and is also stable to treatment with acid, solutions of "avidin" are slowly destroyed. However, the sample tested had been kept in the form of a dry powder.

The data on the interchangeable activities of "avidin" and lysozyme, along with the data obtained by Meyer[11] strongly suggesting that the lysozyme activity of "avidin" concentrates is due to the avidin-biotin complex, place "avidin" in a new light and promise to provide explanations for certain characteristics that have hitherto appeared paradoxical. Thus, György's observations that "avidin" was "toxic" when given orally, while it was therapeutic when administered parenterally,[12] must now be considered in the light of the present findings, which indicate that "free avidin," rather than being "anti-biotin," more likely serves as a biotin-carrier and thus may be more properly termed a "pro-biotin," its so-called "toxic" effect being due to other reasons, such as molecular size, resulting in its non-absorption from the gastro-intestinal tract.

The data reported here, as well as the data obtained by Meyer, point to the need of a thorough reexamination of the literature on lysozyme from various sources that has appeared since its discovery by Fleming in 1922,[13] and also of the literature of other seemingly related products of bacterial and animal origin, such as the various forms of hyaluronidase and "spreading factor."[14] It may be useful at this time to propose as a working hypothesis that "free avidin" is a member of a group of related substances acting as carriers in a system of enzymes in which biotin serves as the prosthetic group.

WILLIAM L. LAURENCE

[10] P. György, C. S. Rose and R. Tomarelli, *Jour. Biol. Chem.*, 144: 169, 1942.

[11] K. Meyer, Personal communication.

[12] P. György and C. S. Rose, SCIENCE, 94: 261, 1941.

[13] R. Thompson, *Arch. Path.*, 30: 1096, 1940.

[14] K. Meyer, E. Chaffee, G. L. Hobby and M. H. Dawson, *Jour. Exp. Med.*, 73: 309, 1941.

THE TOXICITY OF ORALLY ADMINISTERED TANNIC ACID

SEVERAL reports,[1, 2, 3] inspired by the use of tannic acid in burn therapy, have recently appeared describing the hepatotoxic effects of tannic acid. Baker and Handler[1] observed striking hepatic necrosis in rats within 48 hours after tannic acid was either painted on an area denuded of skin or injected subcutaneously. It seemed of interest to determine the effects, if any, of orally administered tannic acid.

The diets used and the results are summarized in Table 1. Twelve rats of the Vanderbilt strain were employed in each group. All animals weighed between 50 and 60 grams initially and the experiments were conducted for 90 days.

TABLE 1

Group	Diet	Final weight	Hepatic damage
1	Ground Purina Chow	240	0
2	" " "	180	0
3	" " " + 1 per cent. tannic acid	188	0
4	" " " + 2 " " "	169	0
5	Synthetic ration[4]	197	0
6	" "	173	0
7	" " + 1 " " "	169	0

The animals in group 2 were pair-fed with those in group 3 and those in group 6 with group 7. The deleterious effect of tannic acid on rat growth appeared to be only due to the animal's dislike for the diet. After 90 days the animals were sacrificed by decapitation and liver specimens from each group were taken for histological examination. In no instance was there evidence of the hepatic necrosis described previously. The gastrointestinal tract appears to be completely impermeable to tannic acid since during the course of the experiment the animals in groups 3 and 7 ingested 100 times the amount of tannic acid which, given subcutaneously, invariably produced hepatic necrosis. The innocuous results of tea drinking, by man, are in accord with these findings.

PHILIP HANDLER
ROGER D. BAKER
DUKE UNIVERSITY SCHOOL OF MEDICINE

[1] Roger D. Baker and Philip Handler, *Ann. Surg.*, 118: 417, 1943.

[2] F. W. Hartman and H. L. Romence, *Ann. Surg.*, 118: 402, 1943.

[3] D. B. Wells, H. D. Humphrey and J. J. Coll, *New Eng. Jour. Med.*, 226: 629, 1942.

[4] The synthetic ration was casein 20, cottonseed oil 15, cod liver oil 5, salt mixture 5, sucrose 55. To each kilogram of this diet were added thiamine 2.5 mg, riboflavin 5 mg, pyridoxine 2.5 mg, calcium pantothenate 40 mg, choline chloride 200 mg.