

TABLE 1

Substance	Amount injected	No. of rats	Total weight of rats (in g.)	Insulin Un. per 100 g. body weight	Per cent. change
Anterior pituitary fractions:					
Control	10	2,900	0.36	...
Fraction 1	518 mg	10	2,200	0.64	+77
Fraction 2	180 mg	10	2,100	0.38	0
Fraction 3	1129 mg	10	2,050	0.26	-28
Fraction 4	63 mg	10	2,350	0.34	0
Prolactin preparations:					
Control	10	2,340	0.42	...
Evans (sheep) ..	400 I.U.	10	2,740	0.00	-100
Evans (sheep) ..	490 I.U.	7	1,550	0.02	-96
White (ox)	300 I.U.	10	2,550	0.08	-81
Riddle (ox)	300 I.U.	10	2,530	0.22	-48
Sterols and stilbestrol:					
Control	10	2,080	0.43	...
Estradiol	12.6 mg	9	2,160	0.54	+19
Estradiol	14.0 mg	10	2,060	0.51	+19
Stilbestrol*	11.2 mg	8	1,710	0.67	+56
Stilbestrol*	14.0 mg	10	1,800	0.62	+44
Progesterone ..	12.6 mg	9	2,730	0.39	-7
Progesterone ..	14.0 mg	10	2,690	0.36	-16
Testosterone ..	14.0 mg	10	2,350	0.29	-31
Testosterone ..	14.0 mg	10	2,870	0.27	-37

* The weight loss is due to toxicity.

SUMMARY

1. Insulinotropic effects were obtained with a protein fraction of the anterior pituitary, with estradiol and with stilbestrol.

2. Varying diabetogenic effects were obtained with highly purified prolactin preparations, with progesterone, with testosterone and with a fraction, probably also protein, from the anterior pituitary.

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CURE OF EGG-WHITE INJURY IN RATS BY THE "TOXIC" FRACTION (AVIDIN)¹ OF EGG WHITE GIVEN PARENTERALLY

RECENTLY¹ it has been shown, in experiments dealing with dietary egg-white injury, that raw or commercial egg white can be supplanted by a particular fraction of egg white (avidin). Previously² the spe-

cific biotin-binding capacity of this substance had been established by the yeast-growth method,³ according to which biotin, in the presence of avidin, becomes unavailable for yeast cells and growth therefore ceases.

It has been found¹ that instead of the 20 to 30 per cent. of raw or commercial egg white needed in the experimental rations to produce egg-white injury in rats, from 0.03 to 0.07 per cent. of avidin is equally effective.

In view of these experimental results the presence of egg-white injury has been explained by the fixation of biotin to avidin in the intestine, the probable non-absorption of the avidin-biotin (AB) complex and its consequent excretion with the feces.

This assumption is borne out by experiments in which the presence of the avidin-biotin (AB) complex in the feces was directly tested. In the absence of avidin, biotin is present in tissues and in feces in free and in bound form, the latter being liberated only by intensive hydrolysis.³ The avidin-biotin complex, on the other hand, is easily split by steaming for a short time (30 to 60 minutes) at 100 C. By the yeast-growth method no difference could be found in the biotin content of feces before and after steaming (4 to 5 micrograms per gram) when rats were fed a normal stock diet. By the same method it was found that rats fed a diet containing cooked egg white plus avidin, however, excreted only negligible amounts of free biotin (in one experiment 0.1 microgram per gram), whereas after the feces had been steamed large additional amounts of biotin became free (4.4 micrograms per gram in the experiment cited).⁴

In order to answer the question how avidin may act in parenteral administration a series of special experiments was required.

Concentrates of avidin were prepared⁵ according to the procedure described by Eakin, Snell and Williams.² The biotin-binding capacity of each concentrate was tested quantitatively by the yeast-growth method.

To learn their effect on egg-white injury the avidin preparations were thoroughly mixed with pulverized, cooked dried egg white in varying amounts in different experiments which corresponded, on the basis of bio-

² R. E. Eakin, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 136: 801, 1940.

³ E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

⁴ By variation of the normal diet and by feeding a pure meat ration we have so far been unable to detect in the feces of rats AB from which biotin could be liberated by steaming unless egg white was also present in the diet.

⁵ The authors wish to express their thanks to Professor R. J. Williams and his collaborators, of the Department of Chemistry, University of Texas, for sending a generous supply of avidin concentrates. Other concentrates were prepared in the Laboratory of the Babies and Childrens Hospital of Cleveland.

¹ The designation of the "toxic" protein of egg white tentatively proposed as *avidalbumin* (P. György, C. S. Rose, R. E. Eakin, E. E. Snell and R. J. Williams, *SCIENCE*, 93: 477, 1941) has been modified by the Texas group to *avidin*, because this protein does not entirely fit into the classification of an albumin. Additional details have now been published (R. E. Eakin, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 140: 535, 1941). In the present and previous studies avidin concentrates have been used, as pure crystallin avidin has not been available.

tin-binding capacity, to the equivalent of from 0.6 to 2.4 gm of egg white per 10 gm of the diet. This mixture was substituted for the original commercial egg white in the experimental diet⁶ used for the production of egg-white injury. Control experiments were carried out with rations containing (1) cooked egg white without the addition of avidin and (2) commercial egg white. Additional special groups of rats fed these two control rations were injected with avidin dissolved in normal saline solution; the daily amounts varied in terms of biotin-binding capacity from the equivalent of 0.3 to 1.2 gm of egg white.

Avidin mixed with the food proved to be "toxic" even in the small doses of one third to one fifth the amount used in previous experiments.¹

Avidin given parenterally, however, did not seem to exert any toxic effect and was unable to prevent improvement in the manifestations of egg-white injury when cooked egg white was substituted for the original commercial egg white in the diet. Pathological symptoms seemed to disappear more rapidly and the gain in weight appeared more extensive in these animals than in the control rats which received the diet containing cooked egg white without the simultaneous injection of avidin. This impression was substantiated by experiments in which rats kept on the original egg-white injury producing diet⁶ were treated, when they were severely "injured," with daily injections of avidin preparations dissolved in normal saline solution. It has been demonstrated that avidin concentrates which are "toxic" when they are given enterally may be of high therapeutic value when they are administered parenterally. The selected examples given in Table I illustrate this conclusion.

TABLE I

Group	Diet containing	Avidin administered	Weight response (gm)	Effect on egg-white injury
A	Cooked egg white	By mouth for 12 days:		
		Rat No. 6344	- 3	Intensified
		Rat No. 6441	- 7	Intensified
		Rat No. 6442	- 7	Intensified
B	Commercial egg white	Parenterally for 12 days:		
		Rat No. 5845	+ 37	Almost cured
		Rat No. 6097	+ 42	Almost cured
		Rat No. 6289	+ 23	Almost cured

An explanation of this paradox must take into consideration the presence of biotin in the avidin preparations. These concentrates contain a large excess of free avidin and, in addition, bound biotin (AB). In one of our preparations the analysis of the daily dose revealed the presence of free avidin in an amount which would inactivate 17 micrograms of biotin as well as the presence of 1.2 micrograms of biotin already bound (AB). It can be assumed that, whereas under

the conditions prevailing in the intestine AB is a stable compound and biotin is thus inactivated, in the parenteral medium a split occurs which liberates the concealed biotin and as a result the biotin acts therapeutically.

The smallest content of biotin found thus far in an avidin preparation which brought about complete cure of egg-white injury in rats when it was administered parenterally was 0.1 microgram in a vehicle of 180 micrograms of avidin preparation. This amount is not far from the therapeutic rat unit (0.04 microgram).⁷

Further experiments are needed to throw light on the special factors which promote liberation of bound biotin from the avidin-biotin (AB) complex under parenteral conditions.

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BIOTIN AND THE GROWTH OF NEUROSPORA

FIVE races of *Neurospora* were found to be biotin-deficient organisms and to require for growth the presence of biotin in the medium. None grew more than slightly in a mineral-glucose solution containing asparagine,¹ or on the same medium solidified with agar which had been purified by extraction with 5 per cent. pyridine and ethyl alcohol.² The addition to these media of peptone, potato extract,¹ agar extract² or pure biotin methyl-ester³ permitted normal growth. Thiamin was ineffective.

The following races were used: *N. sitophila* 56.2 and 56.6; *N. tetrasperma* S₁ and S₂; *N. tetrasperma* J₁, carrying the dominate lethal E; *N. tetrasperma* C₄ and C₅ carrying the recessive lethal d. A wild strain of *N. sitophila*, collected in Bermuda by Dr. F. J. Seaver, was also tested.

Twenty-five ml quantities of a basal mineral-glucose solution containing asparagine in 125 ml flasks were inoculated with small bits of mycelium. A thin mat of mycelium 3 or 4 mm in diameter formed in the liquid within seven days, but no further growth occurred. Sub-cultures into the same medium grew about as well but no better. The basal solution was varied by the addition of thiamin, potato extract, agar extract or pure biotin methyl-ester. The addi-

⁷ P. György, C. S. Rose, K. Hofmann, D. B. Melville and V. du Vigneaud, *SCIENCE*, 92: 609, 1940.

¹ Wm. J. Robbins and K. C. Hamner, *Bot. Gaz.*, 101: 912-927, 1940.

² Wm. J. Robbins and Roberta Ma, *Bull. Torrey Bot. Club*, in press.

³ The biotin methyl-ester was furnished through the courtesy of Dr. Vincent du Vigneaud.

⁶ P. György, *Jour. Biol. Chem.*, 131: 733, 1939.