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 diet[6] 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 diet[6] 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 extract[2] or pure biotin methyl-ester[3] permitted normal growth. Thiamin was ineffective.

The following races were used: *N. sitophila 56.2* and *56.6; N. tetrasperma* S_1 and S_9; *N. tetrasperma* J_1, carrying the dominate lethal E; *N. tetrasperma* C_4 and C_8 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-

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

[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.

tion of thiamin had no effect on the growth of any of these strains. They all grew rapidly in the solutions with potato extract, agar extract or pure biotin. S_1 and S_9 grown together produced abundant conidia and perithecia in these solutions. Mature ascospores were observed in the solutions with potato extract and agar extract but not in those with the pure biotin. 56_2 and 56_6, non-conidial races, produced perithecia and mature ascospores in all these solutions. C_4 and C_8 together produced abundant conidia and perithecia, but no ascospores were formed, which is normal for a mating of these recessive lethal races. J_1 formed conidia and abundant perithecia. A small number of ascospores matured in cultures of this lethal. The Bermuda strain was unisexual and produced abundant conidia. Protoperithecia formed in the solutions with biotin and potato extract. The presence of biotin in potato extract[4] and agar extract[2] has been reported.

The effect of biotin on S_1 and C_8 was studied in agar cultures containing the basal solution solidified with 1 per cent. purified agar. Tubes were inoculated with one drop of a suspension of conidia in distilled water. Both strains grew very little on the purified agar but grew rapidly with the production of abundant conidia when pure biotin, agar extract or neopeptone was added. Higher concentrations of biotin, 0.05 microgram per culture, or agar extract equivalent to 5 per cent. agar, caused a larger number and more rapid development of the protoperithecia. Cultures of C_8 lost their typical lethal appearance and grew like normal *N. tetrasperma* when agar extract, equivalent to 1 per cent. agar, was added, but showed all the features characteristic of the lethal form when agar extract equivalent to 5 per cent. agar was added.

Although all the strains tested were biotin-deficient and grew little or not at all without the addition of that growth substance to the medium, a synthetic medium containing biotin as the sole growth substance was entirely satisfactory for the 56_2 and 56_6 races only. Additional factors of some type appear to be necessary for free production of ascospores by the combinations S_1 and S_9, C_4 and C_8 and the bisexual J_1 race. A detailed report of this work will be published.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A PRECISION FINE ADUSTMENT FOR STANDARD MICROSCOPES

EXPERIENCED microscopists have long maintained that correct interpretation of three-dimensional structures, particularly of biological materials, can only be obtained by continual refocusing. For illustration purposes drawing can suggest the third dimension, but this is not real evidence, since interpretation is involved.

Single photomicrographs involve interpretation since the third dimension is not indicated. However, series of photomicrographs taken with constant differences of focus can show all the changes in appearance that the microscopist sees. Though such series can not be made with the unmodified standard microscope, they can be made with the Graton[1] microscope and with the standard microscope fitted with a lever and a tangent screw. Illustrations made with such a microscope are shown in a paper now in press by Hamly and Watson.[2]

The instrument used in making the above-mentioned illustrations was designed some three years ago, and since then many series of photomicrographs have been made with it. The figure shows part of the Zeiss microscope model #1c (1906) and the modifications made. Most modern microscopes could be so changed; the microscope must have a rigid stand and a fine motion with low lag, smooth operation and low friction.

While the scale indicates 0.1μ divisions, springiness and lag can make small movements meaningless unless certain precautions are taken. They are: (a) the microscope must be moved upward rather than downward by the tangent screw; (b) the microscope should not even be touched during focusing; (c) preliminary visual adjustments should be made carefully until the operator is certain that the principal optical cross section is included in the series. Good series are made with differences of 0.2μ, but this is close to the practical limit caused by residual springiness and lag.

All photographs of the series should be made on the same plate or film, so that all peculiarities of emulsion, development and fixation will be common. Variable exposures can be eliminated by the use of an automatic shutter, or stop watch, provided the source of light does not vary. Series of exposures on one plate or film are easily made in a camera fitted for a sliding plate holder such as the Zeiss Multiplex which the author uses.

The precision motion is not much help in ordinary visual work except in making measurements. How-

[4] Wm. J. Robbins, *Bot. Gaz.*, 102: 520–535, 1941.
[1] L. C. Graton and E. B. Dane, *Jour. Opt. Soc. Amer.*, 27: 355–376, 1937.
[2] D. H. Hamly and J. H. L. Watson, *Trans. Roy. Soc. Canada.* In press, 1941.