

placed raw egg white, perosis did not develop. It was found in a preliminary experiment that all the symptoms were prevented by injecting a biotin concentrate<sup>†</sup> at a level which supplied approximately 3  $\mu\text{g}$  of biotin daily. In the next experiment, crystalline biotin methyl ester<sup>‡</sup> was injected into the breast muscle. Each chick received a total of 13  $\mu\text{g}$  divided into 7 injections during a period which extended from the 12th to the 32nd day. Eight chicks were used in the injected group and 11 uninjected chicks served as controls. The results shown in Table I were observed on the 38th day.

TABLE I.

| Supplement | % of birds showing |            | Avg wt,<br>g |
|------------|--------------------|------------|--------------|
|            | Perosis            | Dermatitis |              |
| None       | 55                 | 82         | 216          |
| Biotin     | 0                  | 38         | 285          |

Feathering was superior in the injected group. The level of biotin administered, which averaged only 0.34  $\mu\text{g}$  per bird per day for the duration of the experiment, was not sufficient to prevent dermatitis completely but was sufficient to prevent perosis.

## 13525 P

### Lens Regeneration from the Iris in Urodeles and its Inhibition by Lens Reimplantation.\*

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In recent publications<sup>1, 2</sup> reviews have been given which point out that the best evidence of lens regeneration from the dorsal iris is limited to a small group of salamanders (*Triturus*). It is of interest to know whether or not all of the *Triturus* group possess this power of lens regeneration. Therefore the effect of lensectomy in *Triturus torosus* was investigated.

<sup>†</sup> Kindly supplied by Dr. Y. SubbaRow, Lederle Laboratories, Pearl River, N.Y.

<sup>‡</sup> Part of the biotin was generously furnished by Dr. Vincent du Vigneaud, and the remainder was purchased from the S. M. A. Company.

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<sup>1</sup> Stone, L. S., and Dinnean, F. L., *J. Exp. Zool.*, 1940, **83**, 95.

<sup>2</sup> Stone, L. S., and Sapir, P., *J. Exp. Zool.*, 1940, **85**, 71.

Stone and Sapir<sup>2</sup> reported that reimplanted lens tissue of the adult *Triturus viridescens* did not survive. Since the reimplant did not persist its inhibitory effect on lens regeneration could not be tested satisfactorily in their experiments. Therefore, in *Triturus torosus* the excised lens was reimplanted to test its capacity for survival. In case it did survive, any inhibitory effect on lens regeneration could be observed.

Lenses were completely removed in 100 eyes of *Triturus torosus* larvae ranging in length from 15 to 25 mm. For histological studies the animals were killed at close stages (2 to 50 days) after operation. The initial stages of lens regeneration were the thickening of the edge of the iris with subsequent depigmentation and separation of the cell layers. These three stages are distinct and progress at about the same rate of development. In all eyes fixed 5 days after operation the thickening of the dorsal iris was observed. At 7 days all specimens showed the first indication of slight depigmentation of the iris and from 9 to 14 days all eyes showed further depigmentation and separation of the cell layers of the dorsal iris. In cases fixed from 14 to 24 days after operation the regenerating lenses varied in developmental rate, especially in the formation of the fiber-forming pole. At 24 days 4 eyes had well-formed isolated lenses. Studies at frequent intervals from 27 to 50 days after operation revealed no new process of lens differentiation but merely one of growth of the regenerated lens.

In the second group of experiments lenses were removed and immediately reimplanted in 42 larval eyes of *Triturus torosus*. The animals were killed from 2 to 50 days after operation. Nineteen of the 42 eyes had reimplanted lenses which appeared normal. These were found in specimens fixed as late as 50 days after operation. In these eyes the dorsal margin of the iris showed no changes that could be interpreted as stages of lens regeneration.

Eleven of the 42 eyes had reimplanted lenses which were cystic. The vacuolated area in 2 extreme cases equaled about one-half the total volume of the lens. In spite of the fact that lenses in this group had areas of degeneration, the dorsal rim of the iris in these eyes showed as in the other group no indications of an attempt to regenerate a lens.

In the remaining 12 eyes of this group the reimplanted lenses were either absent or present as small remnants of lens tissue. Three of these eyes were fixed within a week after operation, in one of which the corneal wound had not closed. Since the removal of the intact lens required a large corneal slit, the absence of these lenses and

possibly others in this group may have been due to the early escape of lens tissue before the corneal wound had finally closed.

In the other 9 eyes in which no lenses were found the specimens were fixed later than one week after operation. Therefore, how long the implanted lens tissue remained in these eyes it is impossible to state exactly. However, the appearance of the dorsal iris may give some clue. For example, in a 15-day case the reaction of the dorsal iris was comparable to that of a 7-day lensectomized eye, indicating that the onset of lens regeneration had been retarded. This delay might have been due to the temporary presence of reimplanted lens tissue. More detailed studies are planned to test further this possibility.

### 13526

#### **Studies of the Action of Sulfonamides upon the Bacterial Cell.**

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The mode of action of sulfonamides upon susceptible bacterial species has been the subject of much study. It appeared to us that one of the most fundamental aspects of this problem was to determine whether active compounds differ from closely related inactive substances in their respective abilities to reach or penetrate the bacterial cell. It was conceivable that p-aminobenzene sulfonamide (sulfanilamide) diffuses into the streptococcal cell (and is concerned in its metabolism) whereas its chemotherapeutically inert isomers, the ortho and meta compounds, do not diffuse into the organism. Since this does not appear to be true, the inactivity of certain compounds cannot be explained by this one fundamental property. In the light of our results diffusion into the organism may or may not be a requisite for a chemotherapeutic agent.

Twenty-liter batches of broth cultures of *Streptococcus hemolyticus*, strain C-203, were grown for 48 hours. The organisms were then collected by means of a continuous flow Sharples centrifuge and washed with 250 cc normal saline 6 to 8 times. The cells were collected after each washing by means of horizontal centrifugation. After final washing the bacteria were dried over P<sub>2</sub>O<sub>5</sub> in a vacuum desiccator. The same procedure was repeated with cultures in broth