Elementary Effect of Arginase in the Weight Physiology of the Mouse

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A series of experiments dealing with the effect of *arginine* and its enzyme, *arginase*, upon growth and repair in the mouse has been the subject of a preliminary report (*1*). It was noted that administration of arginase intraperitoneally resulted in a systemic effect observable in a corresponding weight curve. Further substantiation of this phenomena has been obtained and will be the subject of this report.

Two series of animals have been used. Results from the first, 20 white mice of the Hamilton strain, with 20 controls, were obtained in January 1946. These were normal, hardy white mice. Results from the second series, 20 C3H strain with the same number of controls, were obtained in March 1947. This strain of mice was bred to develop tumors. The procedure upon both groups was identical, with the exception that the arginase used on the first series was obtained from the

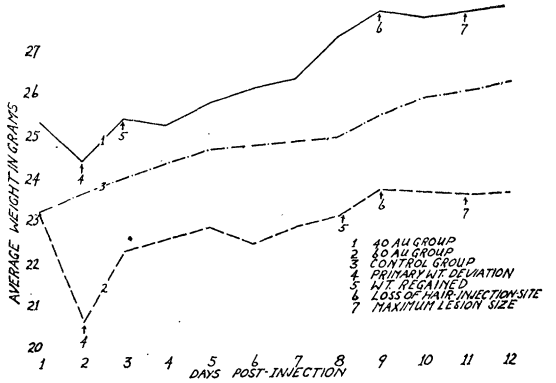

FIG. 1. Hamilton strain.

Biochemistry Laboratories of the University of California, and that used on the second series was prepared in our own laboratories. The same unit dosage was used in both instances.

In the first and second series the animals were divided into groups of 10. One group received 40 AU (arginase units) (*2*) and a second group, 60 AU. Each animal was weighed prior to injection and at the same hour daily thereafter. (Minor exceptions are indicated in Figs. 1 and 2.) The injections were given intraperitoneally in the lower abdominal region.

Although a year elapsed between the two series, similar corresponding curves were obtained; and, in all animals injected, a lesion developed at the sight of injection upon the 8th day.

The weight charts indicate weight in grams and days post-injection of Series I and II, respectively. It will be observed that in the two groups of the first and the second series receiving 40 AU there was a weight deviation in the first 24-hour period of 0.9 gram and 0.8 gram, their injection-day weight being regained between the 3rd and 4th day. The two groups receiving 60 AU showed a weight deviation in the first 24-hour period of 2.6 grams and 2.0 grams, respectively. These did not regain their injection-day weight until late in the 7th or early in the 8th day. The lesion mentioned above developed in all of the injected groups on the 8th day. In the first series, both the 40 AU and the 60 AU groups manifested the lesion by a loss of hair around the injection site, followed in a progressive manner by a complete autolysis of the skin, abdominal musculature, and fascia to (but not including) the peritoneum, reaching this point upon the 11th day post-injection. In the second series, the 40 AU group showed a loss of hair, but further autolysis did not occur. The 60 AU group of this series, however, did develop the complete lesion as described.

The loss of hair on the 8th day is the first indication of any physiological reaction other than the weight change. The denuded surface shows no evidence of an inflammatory process. In the animals that experienced autolysis, the lesion maintained a sterile appearance during its entire progress.

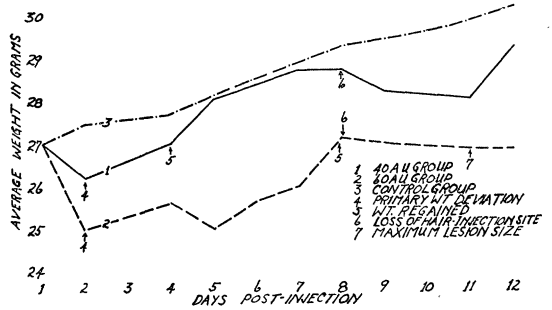

FIG. 2. C3H strain.

No rubor, suppuration, exudate, or swelling was in evidence. Regeneration appeared at the periphery, and the area covered over with a resultant permanent scar and loss of hair. The weight then tended toward normal.

The following conclusions may be drawn:

(1) The AU dosage is proportional to the time necessary for the animal to regain its injection-day weight.

(2) The differential in the primary weight loss of each of the two dosage-related groups indicates that there is a greater ratio of free arginine in the C3H tumor-producing strain.

(3) The greater arginine ratio in the C3H strain requires a slightly higher amount of arginase than the 40 AU used to produce the complete lesion.

(4) Re-establishment of the preinjection arginine-arginase balance in the intercellular fluid begins at the periphery of the lesion where the greatest dilution of the enzyme occurs. While

the injection was made intraperitoneally, the lesion results from an amount of the enzyme spreading about the trauma produced by the insertion and removal of the needle.

(5) The introduction of a suitable amount of arginase into the system buffers the arginine of the body, and, when into the tissues directly, inhibits the action of this substance in normal cell metabolism.

References

1. IRONS, W. G. *J. dent. Res.*, 1946, 25, 497-499.
2. MOHAMED, M. S., and GREENBERG, D. M. *Arch. Biochem.*, 1945, 8, 349-364.

Antibiotic Compound Isolated From the Lichen *Ramalina reticulata*

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Experiments conducted during the period July 1945–January 1947 showed that a crystalline substance isolated from *Ramalina reticulata* had *in vitro* antibiotic activity against a variety of gram-positive organisms and some acid-fast bacteria, including *Mycobacterium tuberculosis hominis*, but not against a number of different gram-negative organisms tested. *In vivo* tests with mice infected with pneumococcus gave negative results, while similar tests with tuberculous guinea pigs showed significant retardation of the progress of the disease (*6*).

Addition of base did not yield simple solutions of the lichen acid, and on the basis of this observation V. C. Barry suggested that the compound isolated might be usnic acid (*1*), shown to be a common constituent of many lichens.

The comparative studies described below were made with a sample of usnic acid received from V. C. Barry and isolated from *Cladonia sylvatica* [L] Harm. emend. Sandst.

The empirical formula (*5*) for usnic acid is given as $C_{18}H_{16}O_7$ [C: 62.79, H: 4.65]. The compound isolated from *Ramalina reticulata* gave the analytical figures [C: 62.88, H: 4.61], as previously reported, in excellent agreement with the above empirical formula. As doubt existed concerning the purity of the parent compound, an attempt was made to prepare a methoxyl derivative with diazomethane. It did not crystallize, but upon distillation in a molecular still gave analytical data [C: 65.75, H: 5.26, OCH_3: 9.50] which fitted the empirical formula $C_{16}H_{14}O_6$ (*6*). However, as the fractionally distilled methoxyl derivative was not crystalline, its homogeneity was also questionable.

A study was made of the homogeneity of the parent compound isolated from *Ramalina reticulata* by the method of countercurrent distribution (*4*). Sixty mg. of the material was distributed in a 24-tube countercurrent distribution machine, using a system containing 20 per cent cyclohexane and 80 per cent benzene as the upper layer and 10 per cent water and 90 per cent methanol as the lower layer, the two phases having first been equilibrated with each other before being used for the distribution. The volumes of the two phases were 12 cc. (upper phase) and 8 cc. (lower phase), giving an operational distribution ratio, K, of 0.41. In Fig. 1 is shown a 24-transfer distribution pattern of the substance. The concentration in each tube was determined by absorption at 284 mμ using the Beckman spectrophotometer. From the nearly complete correspondence with the theoretical curve it can be concluded that the material is apparently homogeneous, and therefore the empirical formula can safely be derived on the basis of the analysis of the original compound isolated rather than the doubtful methoxyl derivative.

The molecular weight of the compound as determined from the methoxyl content in the methoxyl derivative and by an alkali acetone titration of the parent compound was about 310.

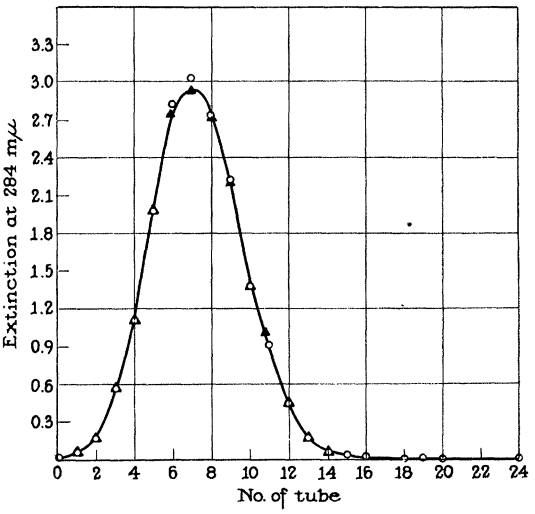

FIG. 1

This value favors a compound with an empirical formula $C_{16}H_{14}O_6$ (M., 302) rather than $C_{18}H_{16}O_7$ (M., 344) (*6*). However, since the acetone titration method employed can be considered valid only to within 5–10 per cent, the value 344 could still be considered possible as the molecular weight for the compound isolated from *Ramalina reticulata*. The coincidental similarity of the molecular weights as determined by the two methods first led the authors to consider the lower empirical formula. The later homogeneity study performed on the parent compound and the additional evidence given below now favors $C_{18}H_{16}O_7$ as the more probable value.

Usnic acid has been reported to melt in the range of 191–205° (*3, 7*). The compound isolated from *Ramalina reticulata* was reported to melt at 193–194° and showed a melting point similar to the compound received from V. C. Barry and isolated from *Cladonia sylvatica*. The melting point of the mixture of the two compounds showed no depression.

The ultraviolet absorption spectrum of the compound from *Ramalina reticulata* (Fig. 2) was similar to that of the compound isolated from *Cladonia sylvatica*. The absorption maxima are at 226–230 mμ and at 284 mμ.

The optical rotation for the compound from *Ramalina reticulata* in chloroform was $[\alpha]_D^{25} = +498°$, which corresponds to the value +495° reported for d-usnic acid (*4, 5*). The compound isolated from *Cladonia sylvatica* gave an $[\alpha]_D^{20} = -20.8°$ in chloroform and was considered to be the racemate of usnic acid with a small admixture of the l-form (*2*).

A study of the adsorption spectrum in the infrared region was made by Konrad Dobriner, of Memorial Hospital, New

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