[11] The latter from Sumner, *Biol. Bull.*, **84**, 195–205 (1943).

[12] Sumner, Amer. Nat., **69**, 245–266 (1935); *Proc. Nat. Acad. Sci.*, **20**, 559–564 (1934); **21**, 345–353 (1935).

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*THE IDENTIFICATION OF GUANINE IN EXTRACTS OF GIRELLA NIGRICANS. THE SPECIFICITY OF GUANASE**

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Sumner in the preceding article (pp. 285–294) has shown that the apparent concentration of guanine in the skin of *Girella nigricans* varies with the albedo of the background against which the fish are kept.

Guanine was identified as a constituent of fish scales about eighty years ago[1,2] and commercial "pearl essence" from this source is believed to consist essentially of free guanine.[3] The existence of guanine in fish scales may therefore be accepted, though with some reservations, since the chemical identification was carried out before isoguanine (2-hydroxy-6-aminopurine) which has properties nearly identical to those of guanine,[4] became available. Within recent years a new series of pigments closely allied to the purines has been studied in detail by Wieland and associates.[5,6] These include isoguanine ("guanopterin")[7] and a number of derivatives of the pteridine (azinepurine) heterocycle. These pigments were isolated first from butterfly wings, but at least one member now is known to exist in fish skin.[8] Therefore, though the identification of guanine as a major constituent of fish skin and scales may be accepted, the absence of similar substances is by no means certain.

The procedure adopted by Dr. Sumner for the determination of guanine, involves a preliminary separation of the material by a precipitation with copper and bisulfite, and finally a colorimetric estimation with phenol reagent. The copper-bisulfite precipitation limits the number of substances which may be present to those of the purine group and substances closely allied chemically[9] but the reaction with the phenol reagent is relatively unspecific.[10] Isoguanine, for example, and some of the pteridines might be expected to be determined as guanine by these procedures. Therefore a quantitative chemical or biochemical procedure was sought which would increase the specificity of the method. Ideally such a procedure, applied to small amounts of material, would distinguish between guanine and all other compounds known to behave like guanine with respect to copper precipitation and the phenol reagent. This would enable one to determine

what proportion of the material estimated as guanine was authentic guanine. Above all one could then determine whether the observed changes in concentration of the substance due to environmental conditions were due chiefly to changes in the concentration of guanine. To a considerable extent a procedure including the use of the enzyme guanase[11] was found to give the desired degree of specificity. This enzyme has been used by Schmidt[12] for the determination of guanine in tissue extracts, but its action on substances closely related to guanine had not been studied previously. Consequently a study of the specificity of guanase action was undertaken.

Guanase was prepared from acetone-dried rabbit liver powder by extraction with water. The extract was purified by treatment with dialyzed iron and finally the enzyme was adsorbed on alumina cream,[13] and eluated with a 0.2 M disodium phosphate solution. An amount of the substance to be tested equivalent to 0.1 mg. of amino nitrogen was incubated 18 hours at 37° with guanase in 0.1 M phosphate buffer at pH 7.8. After the addition of an excess of sodium tetraborate solution, the ammonia which had been

ACTION OF GUANASE ON THE PURINE OF EXTRACTS OF *Girella Nigricans*

	GUANINE FOUND, MG. GUANINE PER G. FISH		
	DIRECT	AFTER COPPER PRECIPITATION	
SAMPLE*	COLORIMETRIC	COLORIMETRIC	GUANASE
Black 3.1	0.386	0.285	0.301
3.2	0.347	0.250	0.245
White 3.1	1.23	1.11	1.08
3.2	1.04	0.91	0.85

* Designations of Dr. Sumner (pp. 285–294).

liberated was distilled into standard 0.02 N acid and the excess acid was titrated. The action of guanase on twelve purines and azine-purines closely related to guanine was studied. Within the limits of experimental error, the enzyme showed no liberation of ammonia with the following substances: adenine, 7-methylguanine, 1, 7-dimethylguanine, 2-amino-6,8-dihydroxypurine ("8-hydroxyguanine"), 2-hydroxy-6-aminopurine ("isoguanine"), 2-amino-6-hydroxyazinepurine, xanthopterin, isoxanthopterin, xanthopterin carboxylic acid and isoxantopterin carboxylic acid. In 10 experiments with guanine an average of 98 per cent of the theoretical amount of ammonia was liberated (range 93–106 per cent). Guanase showed a definite action on 1-methylguanine. In three experiments carried out as above an average of 81.0 per cent (maximum 84.2 per cent) of the theoretical nitrogen was liberated. The action of guanase was demonstrably slower on 1-methylguanine than on guanine itself. With a five-hour incubation period and a somewhat feebler guanase solution, 69 per cent of the theoretical ammonia was liberated from guanine, while 8.5 per cent was being liberated from 1-methylguanine.

The action of guanase on the purine fraction of extracts of *Girella nigricans* then was studied. For this purpose fish were chosen to represent wide differences in apparent guanine content. The results of these experiments are given in table 1. Each extract contained about the same amount of material which reacted with the phenol reagent but was not precipitated by copper and bisulfite and thus was non-purine in character. In each instance the concentration of guanine found by the guanase method was identical with that found colorimetrically within the limits of accuracy of the enzymatic method.

Whereas guanase failed to show a complete specificity in its action, by its use it was shown that isoguanine and a number of purines and pteridines could not be present in the extracts. The failure of guanase to distinguish in all or none fashion between 1-methylguanine and guanine,[14] leaves open perhaps some possibility that the former substance might be present in the extracts, though 1-methylguanine has never been isolated from natural sources. To a considerable extent, this possibility was eliminated by a determination of the substance as the picrate.[15] A solution of the fish purine containing 0.92 mg. total nitrogen, and 0.81 mg. guanine nitrogen as determined colorimetrically was precipitated at a volume of 6 ml. by half saturation with sodium picrate. Titration of the precipitate gave an apparent guanine content of 0.85 mg. of nitrogen. When 1.00 mg. of guanine nitrogen was treated in the same way, 1.02 mg. were found by titration. On the other hand 1.00 mg. of nitrogen as 1-methylguanine gave a recovery of only 0.71 mg. due to the greater solubility of the picrate of this compound.

The results leave little reason to doubt that substantially all the apparent guanine of the extract is authentic guanine, and that the amount of guanine in the fish skin changes with changes in the environmental conditions under which the fish are kept.

* From The Wellcome Research Laboratories, Tuckahoe, New York.

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11 Schmidt, G., *Hoppe Seyl. Z.*, **208**, 185–224 (1932).

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14 It is interesting to note in this connection that in another biological activity, the

germination of spores of *Phycomyces blakesleanus*, 1-methylguanine approaches in activity guanine itself where other methyl guanines and closely related compounds have activities of a lower order of magnitude. (Unpublished experiments with Wm. J. Robbins.) Since methylation in the 1-position would result in fixation of the lactam tautomer, it is perhaps permissible to speculate that with guanase and with *Phycomyces* it is the lactam form of guanine which is active.

[15] Hitchings, G. H., and Fiske, C. H., *Jour. Biol. Chem.*, **141**, 827–835 (1941).

ON SPONTANEOUS MUTATION

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The occurrence of spontaneous mutation in a stock called *px bl* was analyzed. Some of the facts have been reported previously,[1] but there were some errors of interpretation in the preliminary report. The *px bl* line is derived from standard plexus by mutation at the *bs* locus together with an appearance of modifiers for plexation and blistering. In an inbred and closely watched brother-sister culture an "upheaval" (formerly called mass-mutation) occurred, in which mutants at the silver arc and rudimentary loci occurred simultaneously, together with return mutation of *px* and *bs* to wild. A similar upheaval was found twice later in mass culture, the same loci being involved but in part with different alleles. All these mutants and return mutants appeared individually many times later in controlled broods of derivatives of *px bl*, or in in-and-out crosses of *px bl* derivatives. These mutants include a long series of characteristic alleles at the *svr* and *a* loci, repeated mutation at the *bs* and *bb* loci, and further secondary return mutation from + to *px* and of the *svr* and *a* alleles to wild type.

In a significant number of cases, two or more mutants or return mutants, including mutation from one allele to another, occurred simultaneously. This applies especially to the *svr* and *a* alleles. In such cases abnormal sex ratios were frequent. With the exception of these occurrences, the mutation rate was very low in *px bl* and its derivatives. But after in-or-outcrossing this rate rose considerably and also an accumulation of mutants was observed within a few generations after the first mutant had been found. This includes not only mutants and return mutants at the loci already mentioned, but also the rather frequent occurrence of others at the Lobe, dachs, Notch, ebony loci. It is remarkable that in a somewhat small, tentative set of x-ray experiments an unusually high rate of mutation was observed, and among the mutants were alleles of *svr* and *a* as well as at the *bs*, *N* and *d* loci; of course, all lines were checked before the experiments for the absence of such mutants.