Salivary Diastases of the Frog and Toad

ALTHOUGH the presence of diastatic enzymes in the saliva of frogs was originally announced by R. Lépine in 1870 [1] and confirmed by R. J. Junold in 1933 [2], its presence seems so largely to have escaped notice that a fresh investigation using newer techniques is not uncalled for, especially since neither author took the precaution of excluding bacterial enzymes, or made any attempt to determine the precise nature of the reactions involved.

The animals used in the present work were normal adult frogs and toads (no distinction was made as to the sex), which were killed by decapitation using a large pair of bone forceps, regurgitation from the stomach being thereby prevented. Four samples for testing were taken from the mouth of each animal, namely: (1) saline swabbings of the saliva, (2) the whole tongue, (3) scrapings of the mucosa of the palate, (4) the intermaxillary gland. Tissues from several animals were pooled and were incubated at room temperature (*c.* 15° C.) for varying lengths of time. When tested against iodine the red-brown colour characteristic of erythrodextrins appeared in all cases. Bacterial activity was controlled, and it may be safely inferred that such diastatic activity as appeared was not due to bacterial enzymes.

The effect of *p*H on this change from starch to erythrodextrin seems to be nil, since a series incubated at ten different values between 3·5 and 8·0 all gave the brown-red coloration within 30 min. Again, no reducing sugars could be detected in any of the preparations.

When the extracts were incubated at 28° C. no reducing sugars could be detected, even after 18 hr., when starch was used as a substrate; but a positive result was obtained in all cases except the mouth swabbings (which could scarcely have been other than very weak) when glycogen was employed. This complete hydrolysis of glycogen (unlike the starch reaction) is sensitive to changes in *p*H, and can be accomplished only in the range *p*H 4·5–5·5, reduction being complete after 24 hr. under these conditions.

A partition chromatograph, kindly run for us by Dr. J. S. D. Bacon, of the Biochemistry Department of this University, confirmed the above results, and showed that, after 18 hr. incubation at 28° C. with the enzymes contained in pooled tongue and intermaxillary-gland extract, hydrolysis went no further than maltose when starch was the substrate; most of the polysaccharide still remained in the higher dextrin form. With glycogen as substrate a very similar picture was obtained, except that a trace of glucose was also present.

Although the above results point to the presence of a true dextrinogenic salivary enzyme, a further check was provided by testing the activity of similar extracts before and after dialysis. This made it clear that the enzymatic activity of the extracts can be inhibited by the removal of chloride ions and restored by their addition.

Four comparable samples taken from the toad (*Bufo bufo* L.) were incubated for 18 hr. at 28° C. with both starch and glycogen substrates with a result similar to that obtained with the frog material, except that extracts of the tongue and intermaxillary gland of the toad both gave positive results when tested with Benedict solution, and it is presumed that the toad enzyme is more active or more plentiful.

It therefore appears that a true salivary amylase is present in these amphibians, although its functional significance is difficult to understand. The work is being continued with the view of elucidating this problem, as well as the wider questions of the phylogeny of salivary amylases and of the significance of buccal enzymes in general.

E. T. B. FRANCIS
E. A. EISA

Department of Zoology,
University of Sheffield. Oct. 13.

[1] Lépine, R., "Arb. physiol. Anstalt. Leipzig", 113 (1870).
[2] Junold, P. J., Inaug-diss. Leipzig (1933).

A New Biometrical Phase Character in Locusts

THE solitary and the swarming phases of the same species of locusts can be distinguished by coloration and by biometrical characters[1]. Among the latter, the ratio E/F of the elytron length (E) to the posterior femur length (F) has been generally used[2], but no proper statistical investigation of the relative merits of various measurements and ratios exists. Careful measurements of all suitable parts of the body have now been undertaken on samples of the desert locust, *Schistocerca gregaria* (Forsk.), taken from wild populations of a very low density (phase *solitaria*), and from swarms (phase *gregaria*), and the data analysed statistically. None of the twenty direct measurements of body parts proved to be suitable, as the frequency distribution polygons for the two phases overlapped widely. The E/F ratios also showed an overlap which made its use uncertain. Moreover, a comparison of E values in the two extreme phases showed that the elytron-length varied, according to the phase, in an opposite direction in the two sexes, thus:

$$E\ \delta\ gregaria > E\ \delta\ solitaria.$$
$$E\ \varphi\ gregaria < E\ \varphi\ solitaria.$$

The fact that E/F in *gregaria* is nevertheless greater than in *solitaria* in both sexes is due to a relatively greater phase variation in the F values, which partly compensates for the irregularity of the variation in E.

A number of other ratios have been tested; but all either showed an overlap, or were unsuitable for

F/C	*Nomadacris septemfasciata* Serv. Male		*Nomadacris septemfasciata* Serv. Female		*Schistocerca gregaria* Forsk. Male		*Schistocerca gregaria* Forsk. Female		*Locusta migratoria migratorioides* R. & F. Male		*Locusta migratoria migratorioides* R. & F. Female	
	Sol.	Greg.	Sol.	Greg.	Sol.	Greg.	Sol.	Greg.	Sol.	Greg.	Sol.	Greg.
Mean	3·87	3·28	3·99	3·38	3·86	3·11	3·93	3·18	3·64	2·98	3·49	2·86
Standard error	±0·018	±0·015	±0·018	±0·015	±0·019	±0·009	±0·013	±0·012	±0·010	±0·008	±0·011	±0·007
Standard deviation	±0·103	±0·123	±0·107	±0·107	±0·137	±0·094	±0·140	±0·102	±0·106	±0·085	±0·124	±0·077
Difference between means	0·59		0·61		0·75		0·76		0·66		0·62	
t	24·67		25·36		47·68		35·36		52·23		47·04	
d.f.	102		83		258		215		230		246	
P	<0·001		<0·001		<0·001		< 0·001		<0·001		<0·001	