is removed by filtration, as above, and dried at  $92^{\circ}-95^{\circ}$ , after which it is weighed and then incinerated (at about 550°) in a porcelain crucible. From the dry weight is subtracted the weight of the residual ash and the difference, expressed as a percentage (c) of the original weight of the sample, is taken as an approximate measure of the connective tissue originally present. The sum of the three weight percentages (a+b+c) is taken to represent the dry weight of the sample, and the difference between that sum and 100 is taken to represent the original water-content percentage (w).

Representative percentages obtained by means of this technique for samples of longissimus dorsi muscle from two hothouse-grown lambs are shown in the accompanying table, along with some additional percentage values derived from Barbella, Hankins and Alexander's analyses<sup>1</sup> of similar samples from the same individual muscles. The additional values are: P, total protein percentage from Kjeldahl decomposition (corresponding approximately to b+c); F, total fat percentage from ether extraction (corresponding approximately to a); W, total water percentage from acetone extraction (corresponding approximately to w); D, dry-weight percentage derived by subtracting W from 100 (corresponding approximately to a+b+c). Values secured by means of the new technique are shown in **bold-face** type.

	Lamb no. 423	Lamb no. 517
Fat cells (a)	4.3	6.2
Total fat $(\hat{F})$	3.1	4.8
Fiber (b)	17.1	19.1
Connective tissue (c) Fiber and connective tissue	3.5	2.7
(b+c)	20.6	21.8
Total protein $(P)$ Fat cells, fiber and connective	21.8	$\overline{21.2}$
tissue $(a+b+c)$	24.9	28.0
Total fat and protein $(F+P)$	24.9	26.0
Dry weight $(D)$	26.5	27.2
Water, by subtraction (w)	75.1	72.1
Water, by extraction $(W)$	73.6	72.8

These lambs were of different breeds, but both had received the same liberal ration. When killed, No. 423 was 117 da. old and No. 517 was 122 da. old, but their dressed weights were alike (29.5 lbs.). The fatcell percentage (a) was 31 per cent. less for the first sample than for the other, while the fat percentage (F) was 35 per cent. less for the first; the two methods of analysis thus show essential agreement in this respect. But in each case a is greater than F, as might be expected, since a represents intact fat cells, while F represents only extracted fat. For each sample, b + cis in essential agreement with P, a + b + c is in essential agreement with F + P and with D, and w and W are in essential agreement. Finally, it is to be noted that the new technique furnished values for fiber alone (b)

1. Proc. Am. Soc. Animal Prod., 1936, pp. 289-294.

and for connective tissue alone (c), for which no estimates can be derived from F, P, D and W.

The new procedures described above were developed partly in the U. S. Bureau of Animal Industry and partly in the laboratories of zoology and plant physiology of the Johns Hopkins University. For practical advice and criticism the writer is indebted to Dr. E. A. Andrews, Dr. R. P. Cowles, Dr. Hugh C. McPhee, Dr. Paul E. Howe and Mr. O. G. Hankins. Financial aid was received from the U. S. Works Progress Administration.<sup>2</sup> Dr. Burton E. Livingston has helped a great deal in the preparation of this paper.

JOHNS HOPKINS UNIVERSITY

HERBERT BAKER

## A PHOTODYNAMICAL BIOELECTRICAL POTENTIAL

ALTHOUGH a great deal of valuable empirical work is now being done on the clinical and morphological aspect of electrical phenomena in animals, there is need of more information concerning the intrinsic mechanism involved comparable to what is known about the potentials in plant cells.

In the course of an extended study of the effect of temperature, oxygen, ions and heavy water on the potential of frog skin an interesting photodynamical effect has come to light. If a frog skin stained in 0.1 per cent. eosin is exposed to strong light from a carbon arc a striking and sudden increase in potential results. The skins were from the belly region tied in holders of 2 to 5 cc of stained Ringer's solution leading through

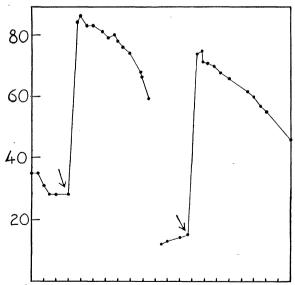


FIG. 1. Increase in potential of two frog skins transferred to Ringer's solution containing eosin and radiated (at arrows). Ordinates: e.m.f. in millivolts (outside surface is negative). Abscissae: time in ten-minute intervals.

Ag-AgCl electrodes to a Type K potentiometer. The light was passed through ice water to avoid a heating effect. The rise in potential in nine skins was 28 to 86, 22 to 52, 15 to 75, 0 to 52, 20 to 50, 24 to 53, 14 to 32, 1 to 45 and 17 to 41 millivolts (see Fig. 1).

The photodynamic effect probably involves the oxidation of membrane proteins, as in muscle,<sup>1</sup> which might provide the slow colloidal anion capable of setting up a diffusion potential with a fast cation like K. Thus the radiated eosin has an effect opposite to that of heavy water.<sup>2</sup>

> T. CUNLIFFE BARNES HAROLD L. GOLUBOCK

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

## THE SPECIFICITY OF PEPSIN ACTION

THE various enzymes which attack genuine proteins and which therefore are designated proteinases exhibit striking differences in the specificity of their chemical action. The clearest demonstration of these differences in enzymatic specificity has been obtained by means of synthetic substrates. Such substrates have recently been described<sup>1</sup> for all the known types of proteinases, with the exception of pepsin.

In this communication we wish to report the finding of a synthetic substrate for swine pepsin. Carbobenzoxy-*l*-glutamyl-*l*-tyrosine is extensively hydrolyzed in the presence of pepsin with the formation of carbobenzoxy glutamic acid and tyrosine; under our conditions the hydrolysis attained 70 per cent. in 3 days. This enzymatic hydrolysis occurs at pH 4. At the generally accepted pH optimum of pepsin—pH 2—, a hydrolysis of only 10 per cent. of the synthetic substrate was observed. Once recrystallized pepsin is more effective than a good commercial preparation.

The availability of synthetic substrates for pepsin makes possible a study of the specificities, homogeneity and kinetics of pepsin preparations from various animal species.

> Joseph S. Fruton Max Bergmann

THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK, N. Y.

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A SYSTEM FOR NUMBERING LABORATORY ANIMALS

THERE are several systems in use for numbering experimental animals, and the following one, which makes use of permanent marks, has certain advantages over many of them.

The marks consist of notches, holes and slits in the ears, clipped toes and clipped tails (some species). Notches (made by scissors) in the right ear are taken as units and notches in the left ear as tens. Two positions on the ear are selected for the notches, the anterior margin or front portion, and the posterior margin or back portion. The notches give a continued sequence from 1 to 99 (see Table 1).

TAE	BLE	1

Number of notches and position on margin	Right ear	Left ear
	No. of animal	No. of animal
1 front (anterior margin)     2 front """     1 back (posterior margin)     2 back """     1 front—1 back     2 "1"     2 "2"     2 "2"     2 "2"     2 "2"	123456780	$ \begin{array}{r}   10 \\   20 \\   30 \\   40 \\   50 \\   60 \\   70 \\   80 \\$

Animal number 11 would have 1 notch placed in the anterior margin of the left ear and 1 notch placed in the anterior margin of the right ear.

<sup>1</sup> A. J. Kosman and R. S. Lillie, Jour. Cell. and Comp. Physiol., 6: 505, 1935. For additional references of. A. J. Kosman, Jour. Cell. and Comp. Physiol., 11: 279, 1938. The series is extended beyond 99 by punching holes in the ears. One hole in an ear designates animal No. 100 and nine holes designates No. 900.

The toes can be clipped off according to some plan and the readings combined with those from the marks in the ears. The toes of the animal are numbered clockwise when the animal is held by the back with its head up and its feet toward the worker. This is done by considering the leftmost toe on the right forefoot, analogous to the little finger on our right hands, as No. 1 and then counting the toes on the forefeet from left to right (clockwise). The toes on the hind feet are numbered from right to left (clockwise). Each toe would represent a thousand and therefore, if the No. 1 toe were clipped it would be animal No. 1,000. If the animal has 18 toes and the left one on the right hind foot were clipped, the animal would be No. 18,000. It is easy to select a combination of toes which will total 49,000 without incapacitating the animal. Then the total number obtained by combining the notches and holes in the ears and the clipped toes is 49,999.

A straight slit, placed in the tip of the right ear so that it does not pass through one of the holes, indicates No. 50,000 and a straight slit in the tip of the left ear signifies an additional 50,000.

<sup>&</sup>lt;sup>2</sup> T. C. Barnes, SCIENCE, 83: 506, 1936.

<sup>&</sup>lt;sup>1</sup> M. Bergmann, J. S. Fruton and H. Pollok, SCIENCE, 85: 410, 1937.