

same type of stainability as does gelatin, namely: staining acidophilic below a certain pH value; and basophilic above a certain pH value, forming the following cell system:

acid — buffer sol.	fatty acid amine mixture in oil shaken with acid acidophilic	shaken with alkal. sol. basophilic	alkal. buffer sol. +
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This shows that the negative pole is on the side of the acidophilic mixture in this case, just as in the case of gelatin and in olive oil mixtures with oleic acid or amylamine. Experimental details about this work will be published soon.

We see, therefore, that stainability and e.m.f. show the same relation in the case of proteins and of fats as well. It has not been possible so far to set up artificial systems composed of proteins which differ in their water-immiscible constituents, the reason being simply that proteins which dissolve a water-insoluble acid are not well known. We expect, however, to overcome this technical difficulty by combining in a cell system proteins containing preferably acid groups with those containing preferably basic groups. It is too early as yet to predict the outcome of such experiments, but if they are possible, they will very likely also reveal the same relation between stainability and e.m.f. which has been found in almost all other cases. *Hence none of our experiments prove that fats exclusively are the cause of bioelectricity.* Proteins might be used in the place of fats in every instance if the present technical difficulties can be overcome.

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An In Vitro Test to Indicate Basophilic or Acidophilic Character of a Dye.

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It has been found¹ that eosin, an acidophilic dye, is taken up by a fat mixture containing an oil soluble base, while a basophilic dye,

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¹ Beutner, R., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 44.

methylene blue, dissolves in a fat mixture with an oil soluble acid. The fat mixture with a base is electrically negative against the mixture with an acid.

Considering the general character of these findings, it seems probable that the same conditions hold also in tissues; hence our model experiments are suitable for explaining that relation of stainability to electromotive forces which has thus far been found in tissues. To corroborate this finding we have performed the following comparative experiments. Forty-three different dyes were used for testing the differential stainability of artificial mixtures such as those mentioned which contain either oleic acid or an amine, and this was compared with the stainability of white blood cells. It was found in each case that a dye which was taken up, exclusively or preferably, by a non-aqueous solution of a higher fatty acid, produced a differential stain on the nucleus of white blood cells. On the other hand, any dye with a preference for a mixture containing a fat soluble base preferably stains the cytoplasm and leaves the nucleus unstained, or but slightly stained.

Many nuclear stains like methylene blue are water-insoluble bases. Evidently, they are taken up by a solution of oleic acid in a suitable solvent, forming, *e. g.*, methylene blue oleate, which is oil-soluble and hence stains. However, there are also nuclear stains which are not bases, *e. g.*, *hematoxylin*, a widely used nuclear stain which has all the characteristics of an acid. It was of special importance to test the type of oil solubility in this case. One might have expected that the acidic hematoxylin was preferably soluble in an amine or alkaloid oil mixture, like other acid dyes, *e. g.*, eosin. Of course, this would have been in disagreement with its biological staining power, and would have tended to show the uselessness of our oil mixture as a biological standard of comparison. The experiment showed that this expectation was not correct. Hematoxylin was found to be taken up by the oleic acid oil mixture and not by the amine mixture. Hence, in this case too, the test tube experiment with oil mixtures furnishes decisive evidence as to the biological staining power; one could foretell that hematoxylin must be a nuclear dye in spite of acidic properties in aqueous solution. Possibly hematoxylin has basic properties in oil solution.