solution, with the exception of sodium, the halogens and sulfur.

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THE NEPHELOMETER IN MYCOLOGY

THE use of the nephelometer in the biological sciences is not new. It has been used to measure the growth of bacteria in nutrient solutions and has been adapted to the determination of protein, using sulfosalicylic acid as a precipitating agent. A method of determining nitrogen as ammonia, using a modified Nessler's reagent, has been worked out for extremely dilute solutions of ammonia or ammonium salts.

It occurred to the writer that a water culture of a fungus such as oidium could be determined by this method, provided the hyphae were broken up sufficiently. A culture of oidium in a dilute nutrient solution was shaken up in a shaking machine for various lengths of time and with the addition of different acids and bases. Attempts to use glass beads and clean sand as aids in the breaking up process failed because of the colloidal silica formed by the rubbing together of the particles.

Best results were obtained by using a glass-stoppered, wide-mouthed bottle approximately four times as high as the diameter and fitted inside with a cone-shaped roll of nichrome gauze. This cone should be tall enough to have the tip pressed upon by the glass stopper and thus prevent its shaking about. The bottle should be not over one third or one fourth full of the culture and should be shaken rapidly for at least twenty minutes. The culture being dashed back and forth through the wire gauze at an angle is torn to pieces small enough to stay in suspension for a considerable time. The culture should be neutral as even a slightly acid solution attacks the wire under these conditions and a colored solution results which is useless for a nephelometric determination. It may be found desirable to remove the gauze cone and add sufficient concentrated sulfuric acid to make a 10 per cent. solution and again shake for five minutes. The increased specific gravity of such a solution will help to keep the particles from settling out. Every effort must be made to keep any turbidity other than that due to the fungus from developing. If any calcium was used in the nutrient solution the addition of sulfuric acid will cause a very objectionable precipitate. In any case a part of the sample should be filtered and the filtrate run as a blank.

If it is merely desired to compare various solutions, one of them may be used as a standard, or a suitable standard can be made by adding one drop of saturated silver sulfate solution to 200 cc of distilled water and adding just enough dilute HCl to completely precipitate the silver. This solution darkens after a time but is good for several readings.

By observing the precaution necessary to successful determinations with a nephelometer the writer has found that the readings are proportional to the amount of growth present in the solution.

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AN ACCURATE METHOD OF TAKING READINGS ON EXCEEDINGLY LOW ROCK DIPS

IN regions of nearly horizontal bedded rocks it is practically impossible to make accurate dip readings with a Brunton compass where the dip is less than fifty feet per mile. It is however often essential in deciphering low structures to get dependable results quickly in the field. The procedure described below was found to meet this requirement very satisfactorily.

Tie a long nail on one end of 100 feet of fish-line. Drive this nail into the bed of rock of which the dip is to be read. Stretch the fish-line across the rock face. Usually a 50-foot exposure or a 25-foot exposure can be had. Such lengths facilitate calculation. Mark the fish-line at 25, 50 and 75-foot intervals by tying on pieces of red yarn or silk thread. Have a small line-level such as is commonly used by carpenters placed over the center of the line and raise the line until the bubble reads level. Then with a carpenter's 6-foot folding rule, note the dip and make the calculation.

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SPECIAL ARTICLES

THE ACTION OF GLUTATHIONE AND HEMOGLOBIN ON THE GROWTH OF FIBROBLASTS IN VITRO

FOR some time it has been known that fibroblasts and epithelial cells proliferate indefinitely in vitro in a medium composed of equal parts of plasma and embryo juice.[1] If the factors responsible for the unlimited growth of tissues in such a medium could be

[1] A. Carrel, "Artificial Activation of the Growth in Vitro of Connective Tissue," *J. Exp. Med.*, 17: 14, 1913; "Tissue Culture and Cell Physiology," *Physiological Review*, 4: 1, 1924; "Les Milieux Nutritifs et leur Mode d'Emploi dans la Culture des Tissues," *C. R. de la Soc. de Biol.*, 96: 603, 1927; Fisher, A., "A Three Months Old Strain of Epithelium," *J. Exp. Med.*, 35: 367, 1922; A. Carrel and A. H. Ebeling, "The Multiplication of Fibroblasts in Vitro," *J. Exp. Med.*, 34: 317, 1921; "Survival and Growth of Fibroblasts in Vitro," *J. Exp. Med.*, 38: 487, 1923.

known, it seems probable that much could be done to accelerate the normal processes of repair. Such knowledge would also assist us in understanding the mechanism of the development of malignant tumors. As a means to these ends it seemed desirable to synthesize, if possible, a medium of known chemical composition in which the indefinite proliferation of connective and epithelial tissues would take place. The first step in this direction was made with the discovery that fibroblasts and epithelial cells cultivated in proteoses from certain proteins multiplied extensively, although for a limited period.[2] It was shown later that peptones and the lower fragments of the protein molecule also contribute to the nutrition of fibroblasts.[3] Recently a pure strain of sarcomatous fibroblasts was found to proliferate slowly for a period of fourteen to twenty days in an artificial medium composed of Tyrode solution, nucleic acid, glycocoll and the peptic digestion products of either casein or crystalline egg albumin.[4] Normal fibroblasts in pure culture also multiply for a limited time in this medium. The rate of growth, however, decreases with time, and death of the cells follows. One could attribute the deficiency of this medium in part, at least, to the absence of substances required for the functioning of the respiratory mechanism of the cell. It was also reasonable to suppose that a given oxidation-reduction potential might be as important for cell multiplication as a definite osmotic pressure or hydrogen-ion concentration. Therefore, a study was made of the effect on the nutritive value of this medium of adding to it, (1) ash of liver, (2) glutathione and (3) hemoglobin. The glutathione used in these experiments was very kindly furnished for this purpose by Dr. Carl Voegtlin, of Washington, D. C. Ash of liver was employed because it contains both iron and copper and has been shown to be beneficial in the treatment of anemia of rats.[5] Also it has been found that the peptic and tryptic digests of liver furnish all the elements essential for the complete nutrition of sarcomatous fibroblasts.[6] For this work a pure strain of sarcomatous fibroblasts of rat Sarcoma No. 10 of the Crocker Foundation was used, and also a pure strain of normal fibroblasts of the rat. The colonies were cultivated in Carrel "D-3" flasks.

Practically no effect on the area of growth of sarcomatous fibroblasts was obtained by adding either ash of liver or glutathione to the artificial medium of casein digest, glycocoll and nucleic acid.[7] However, the condition of the cells was slightly improved and an increase in the thickness of the growth occurred. When both substances were added to the artificial medium, there was a small but very definite increase in the rate of growth especially noticeable in the second passage, *i.e.*, after about twelve or sixteen days. They also caused a marked improvement in the condition of the cells. When hemoglobin was added to the mixture of casein digest, glycocoll and nucleic acid, there was also a slight increase in the nutritive value in this mixture for sarcomatous fibroblasts and a slight lengthening of their period of survival. Glutathione, ash of liver and hemoglobin were then all added together to the artificial medium. The mixture of these three substances with casein digest, glycocoll and nucleic acid was found to have an astonishing effect on the multiplication of sarcomatous fibroblasts. The growth of new tissue was approximately 100 per cent. greater in the first passage than that in the original medium of casein digest, glycocoll and nucleic acid, and was as great as that produced by embryo juice. Moreover, the same rate of growth continued for five passages, or about forty days. Toward the end of this time, the growth became thinner and the condition of the cells was not as good as that of the controls cultivated in embryo juice. The central fragment of the colony was surrounded with dead cells and some dead cells were scattered throughout the culture. This might be accounted for in one of three ways: (1) some essential substances are still lacking in this artificial medium; (2) the medium contains some substance which is toxic or (3) the proportion of the different constituents of the medium to each other is not correct. Subsequent experiments demonstrated that equally good results were obtained when only glutathione and hemoglobin were added to casein digest, glycocoll and nucleic acid. The ash of liver seems to have no effect when hemoglobin is also used.

[2] A. Carrel and L. E. Baker, "Action des Proteoses sur la Proliferation Cellulaire," *C. R. de la Soc. de Biol.*, 95: 359, 1926; "The Chemical Nature of Substances Required for Cell Multiplication," *J. Exp. Med.*, 44: 503, 1926.

[3] L. E. Baker and A. Carrel, "Nitrogen Metabolism of Normal and Sarcomatous Fibroblasts in Pure Culture," *J. Exp. Med.*, 1928 (in press).

[4] L. E. Baker and A. Carrel, "The Effect of Digests of Pure Proteins on Cell Proliferation," *J. Exp. Med.*, 47: 353, 1928; A. Carrel, L. E. Baker and A. H. Ebeling, "The Effect of Certain Pure Chemical Substances on the Multiplication of Sarcomatous Rat Fibroblasts," *Archiv f. exp. Zellforsch.* 5: 125, 1927.

[5] E. B. Hart, H. Steenbock, J. Waddell and C. A. Elvehjem, *J. Biol. Chem.*, 77: 797, 1928.

[6] L. E. Baker and A. Carrel, "Effect of Liver and Pituitary Digests on the Proliferation of Sarcomatous Fibroblasts of the Rat," *J. Exp. Med.*, 47: 371.

[7] In all cases the tissues were embedded in a coagulum of chicken plasma from which the serum was removed by washing with Tyrode solution. The artificial medium was made up in Tyrode solution and used as a fluid medium on top of the coagulated plasma.

It is possible that the hemoglobin fulfils the same function as the ash, only more efficiently. When normal fibroblasts of the rat were used in place of sarcomatous fibroblasts, they also were found to proliferate more rapidly when glutathione, ash of liver and hemoglobin were present in the artificial medium, but the results were not as marked as were those with sarcomatous fibroblasts. As has been observed before, normal cells are more subject to the deficiencies of artificial media than are sarcomatous fibroblasts. It seems probable that hemoglobin and glutathione function not only by regulating the respiration and oxidation or reductions within the cell but also by causing a desirable oxidation-reduction potential in the medium. This hypothesis will be tested in the near future. Without doubt, they furnish constituents which are essential as foods for the synthesis of cell protoplasm.

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ZOOPHILOUS MOTHS[1]

THE night-flying moths in the vicinity of Iguazú Falls (Misiones, Argentina) have a perversion of taste remindful of the perverted food habits of the keas of New Zealand.

The writer, in company with Dr. Eduardo Del Ponte, first became acquainted with their abnormal habits while investigating the anopheline mosquitoes of the Rio Alto Paraná for the Departamento Nacional de Higiene de Argentina (June, 1927). Mr. Adams, manager of the hotel at the Cataracts, related to us that swarms of night-flying "butterflies" caused his horses considerable annoyance by "getting" into their eyes, and the consequent irritation sometimes resulted in temporary blindness. We thought they could not be true butterflies, as these are diurnal; neither could we believe they were moths, as it seemed incredible that they could have such habits. However, Mr. Adams insisted the insects were like butterflies.

During our stay we used two of Mr. Adams's horses as bait to attract mosquitoes. The first evening a moth was seen to alight on the lower eyelid of one and immediately, as we could observe by means of flashlights, it extruded its proboscis and began to feed upon the secretions. We were unable to capture the moth as the horse frightened it away by winking its eye. At this time of year (the winter season) moths were very scarce and no more were seen. However, our observation confirmed Mr. Adams's statement that the insects were butterfly-like, and also gave the clue to the correct group of insects to be implicated, namely, the Geometridae.

The following October, in company with Mrs. Shannon and Señor Marcos Reisel, I was again making anopheline investigations at the Iguazú Falls and as the season was favorable, we were able to collect a number of moths off the horses. A surprising thing is that a large number of species, representing several families, including even the Sphingidae, are attracted to the horses.

Dr. H. G. Dyar (National Museum) kindly identified the moths and their names are given below:

	LIST OF SPECIES
Pyralidae:	*Pyrausta* sp.
Notodontidae:	*Crinodes beskei* Hubn.
Geometridae:	*Pergama polygonaria* H.-S.
	Pergama speciosata Gn.
	Pergama pumaria Feld.
	Meticulodes xylinaria Gn.
	Pero stolidata Gn.
	Pero maculicosta Warr.
	Dichromatopodia deflexa Warr.
	Pterocypha tabascana Schaus.
Sphingidae:	*Xylophanes tersa* L.

Upon our return to Buenos Aires, we related our experience to Dr. Carlos Bruch (local entomologist) who then told us that in 1904 he received a letter from a friend in Paraguay (eastern part?) saying that his horses were being troubled by "mariposas" coming to their eyes, producing thereby great irritation. This is the only additional record so far obtained.

The reason for this perversion of habit is not plain. Mr. Adams suggested that possibly the moths were attracted by the light reflected from the eyes of the horses. The fact that we saw numerous moths sucking the eye secretions, and also visiting other parts of the body and endeavoring to feed on the perspiration, indicates clearly enough that they desire the food, salt or moisture contained therein. The last factor appears of small consequence as the region is well supplied with moisture by ample rainfall.

We are also left to wonder why so many kinds of moths choose horses (and probably cattle as well) as a source of nourishment. But this in itself may help to explain why they have developed their anomalous taste. Possibly, the explanation lies in the fact that, in spite of the superb development of plant life in the region, flowers (the usual source of food for moths) are very scarce and, therefore, the moths are forced to seek food elsewhere. This is corroborated to some extent by finding many kinds of flower-feeding flies and bees, existing in the region, feeding

[1] Contribution from the Instituto Bacteriologico, Buenos Aires.