therewith. It is also possible to make legible projection material by simply typing the matter as one would a mimeograph stencil, on a blank piece of film. The ribbon can be left in, but it does not add greatly to the legibility and will smudge, as will a carbon under these conditions.

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SPECIAL ARTICLES

SELECTION WITH THE MAGNET AND CULTIVATION OF "RETICULO-ENDOTHELIAL" CELLS

THE supposition that certain highly phagocytic cells situated along the sinuses of the liver, spleen, bone-marrow, lymph-nodes and other organs have activities in common besides phagocytosis and constitute a physiological system, the "reticulo-endothelial system," has led to much discussion and experimentation. A host of functions, among them those of forming antibodies and bile pigment, are attributed to the cells in question. These attributions have been the easier because only oblique methods of test for them have been available.

Von Kupffer, the discoverer of the cells in the liver now classed as "reticulo-endothelial," observed that after the cells have taken up particulate material from the blood flowing by them, a greater or less proportion lose their hold on the capillaries and come away into the stream, new ones being provided by a proliferation and differentiation of the vascular endothelium. This happens irrespective of the character of the material phagocyted. We have taken advantage of the phenomenon to procure and cultivate the Kupffer cells.

A suspension of highly magnetic iron particles (the gamma ferric oxide of Baudisch and Welo[1]) in 7 per cent. gum acacia solution is injected into the circulation of a rabbit (or dog) on several successive days; and after two or three further days have elapsed—to give time for the particles ingested by blood leukocytes to be deposited—the animal is anesthetized and fluid is run directly through the liver, at first under low pressure to wash away the blood, then under high, with intermittent obstruction of the outlet tube and kneading of the liver to loosen and flush out the Kupffer cells. Warm Tyrode solution with 1/8 per cent. of gelatin for protective purposes[2] has proved as satisfactory a fluid as homologous serum. The Kupffer cells containing iron are separated from other elements by means of an electromagnet, past which the suspension is slowly run, "washed" with gelatin-Tyrode solution while still held by the magnet, and plated in a culture medium consisting of this fluid, plasma and serum.

When first obtained in serum or Tyrode's solution and studied in the warm box the Kupffer cells have the general character of clasmatocytes, but they exhibit in addition special traits which distinguish them from those phagocytic elements of the spleen and of old inflammatory exudates which are supposed like them to be components of the "reticulo-endothelial system." It is plain that this "system" consists of elements differing from one another to no inconsiderable extent.

Kupffer cells proliferate *in vitro* despite an initial content of iron particles that is often large; and they retain their specialized character. Since this is the case experiments with cultures should throw light on the functions of the cells. Such experiments are under way.

It is obvious that the magnet can be utilized for the selective separation of the phagocytic cells of organs other than the liver.

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[1] Provided through the generosity of Dr. Oskar Baudisch.

[2] Peyton Rous and J. R. Turner, *Jour. Exp. Med.*, 23: 219, 1916.

RELATION BETWEEN OXYGEN TENSION AND PROTEIN SYNTHESIS IN CERTAIN TISSUE EXTRACTS

IN previous work[1] we showed that a marked decrease in oxygen tension below atmospheric tension under otherwise constant conditions of pH, temperature, concentration of substrate, etc., increases the degree and the rate of proteolysis in certain normal and malignant tissues. These results suggested experiments designed to show whether oxygenation of digests of tissues containing suitable protein split products would or would not result in enzymatic protein synthesis. Concentrated extracts of the following tissues in phosphate buffer (pH approximately 7.0) were used: Voluntary muscle of albino rats and rabbits, Jensen rat sarcoma and Walker rat carcinoma 256. The extracts were subjected to a preliminary period of digestion in an atmosphere of purified nitrogen, toluene being added to prevent bacterial growth. The digests were then treated with a current of purified oxygen from 2 to 4 hours. The mixtures were finally allowed to digest again in an atmosphere of nitrogen. The protein content was deter-

[1] Carl Voegtlin and M. E. Maver, Public Health Reports, 47: 711, 1932; M. E. Maver, J. M. Johnson and Carl Voegtlin, Public Health Reports, in press.

mined by precipitation with trichloracetic acid,[2] and the total nitrogen was estimated in the precipitates by the micro-Kjeldahl method.[3] Simultaneous estimations were made of reduced and oxidized glutathione, the former being estimated by the iodometric method, using nitroprusside as end-point indicator, the latter being titrated after reduction with bismuth tartrate and H_2S. The total SH (SH glutathione, plus protein SH groups) was determined by iodine titration, and the pH by the glass electrode.

We found that oxygenation of digests of the muscle results in a relatively rapid protein synthesis, representing an increase of from 10 to 20 per cent. of protein nitrogen in terms of total nitrogen. Similar results were obtained with digests from the living portion of the Jensen sarcoma and Walker carcinoma, and likewise in a digest composed of papain plus fibrin and SH glutathione. Our results with these extracts indicate that the conditions favoring synthesis of proteins are (1) a relatively high oxygen tension; (2) a hydrogen-ion concentration not far removed from neutrality; (3) a relatively high initial concentration of SH groups attached to protein or glutathione which can give rise to a relatively high concentration of disulfides; (4) a sufficient concentration of suitable protein split products.

Small amounts of $CuSO_4$ added to the digests immediately before the oxygenation is begun may cause a temporary acceleration of protein synthesis, which, however, is followed by a resumption of protein cleavage.

These results obtained *in vitro* are of interest, as they suggest that variation in the oxygen supply of the tissues under physiological and particularly pathological conditions may exert a controlling influence on the equilibrium between cleavage and synthesis of tissue proteins. It may be that an inadequate O_2 supply to certain portions of malignant tumors favors necrosis and digestion of intracellular protein. On the other hand, in portions of tumors which are well supplied with oxygenated blood, conditions may be favorable for protein synthesis and therefore tissue growth. Further work is required before these deductions can be applied to other normal and malignant tissues. A detailed report of this investigation will be published in the *Journal of Pharmacology and Experimental Therapeutics*.

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[2] Seibert, *Jour. Biol. Chem.*, 70: 265, 1926.
[3] Koch and McMeekin, *Jour. Amer. Chem. Soc.*, xlvi: 2066, 1924.

THE UNIQUE NUTRITIONAL ORGANS IN THE EMBRYOS OF THE TOP MINNOWS OF THE MEXICAN PLATEAU

DURING Cretaceous times the Mexican Plateau was isolated to some extent by the development of high volcanic ridges on the east, south and west and by desert on the north and the northwest. A fauna has developed here which is quite unique in some animal groups, and this is especially true in the case of the teleost fishes. While there has been some migration into and out of the plateau, for the most part the species upon the plateau do not exist elsewhere. In one instance, a family which is represented in North America elsewhere by a single species has bloomed in the isolation provided by plateau into many species.

It has been pointed out by Hubbs that most of the top minnows of the Lerma Valley should be separated into a single family, the Goodeidae. The classification is based principally upon the structure of the teeth and fin peculiarities. The family, as revised by Hubbs, contains the Genera Skiffia, Lermicthys, Charcodon, Balsadicthys, Goodea, Chapalicthys, Zoogoneticus and Girardinicthys. A study of the reproduction of seven of these genera reveals peculiarities that separate the group radically from all other top minnows and several features which are unique and undescribed. These features are the subject of this brief article, which is to be followed later by a more extensive illustrated description.

The course of reproduction in the lowland type of killifish (Gambusia, etc.) is characterized by the following features:

(1) Fertilization is accomplished by the transfer of spermatophores from male to female by means of a gonopod. Fertilization is internal. The sperm are stored by the female and serve for several succeeding broods of young.

(2) The ovarian follicle builds up a large yolk mass which is the sole means of nourishment for the embryo.

(3) The embryo remains within the ovarian follicle until hatched. It is then extruded into the intra-ovarian space and is born almost immediately. The yolk mass is almost entirely absorbed at birth.

(4) The gonads of the embryo are quite undeveloped at birth, no differentiation of ovary or spermary being discernible.

The contrast as shown in the reproduction of the highland type, Goodeidae, is indicated as follows:

(1) There are no spermatophores and there is no true gonapod in the male. In all genera, however, the anal fin has some short stiff rays at the anterior margin. Fertilization is internal, but apparently there is no storage of sperm.

(2) The ovarian follicle builds up a very small yolk