result of an upset in the binding mechanisms of the tissue amines. Thus, the adrenal cortical secretion, which is mainly corticosterone in the rat and thus glucocorticoid in nature, may exert a functional control over the tissue-levels of both histamine and 5-hydroxytryptamine.

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Adaptation to Serum-free Medium by a Phagocytic Cell Strain derived from a Murine Lymphoma

The adaptation of mammalian cells to serum-free media in tissue culture has been a subject of considerable interest in recent years. Cells grown in such media would be free from the effects of serum antibodies and other serum inhibitors of micro-organisms, and, therefore, should be suitable for long-term studies of intracellular bacteria as well as rickettsia and viruses. Such cells might also be useful in nutritional studies in tissue culture and in evaluating the effects of heterologous sera on cells in culture.

We have recently been studying *Mycobacterium lepraemurium* in tissue cultures of a continuous line of neoplastic highly phagocytic cells, derived by Dawe and Potter[1] from a lymphocytic neoplasm (strain *P*388) of *DBA*/2 mice. This cell line was originally isolated in a medium containing 40 per cent human serum and seemed to require serum for growth.

In order to avoid contact of the murine leprosy bacilli with serum, the cells were transferred to a serum-free maintenance medium prior to infection and remained in this medium throughout the experiments. Since the cells rapidly deteriorated in the complete absence of protein, a maintenance medium consisting of 20 per cent autoclaved fatless milk and 80 per cent medium No. 199 was selected. This medium has recently been described by Baron and Low[2] in virological studies and seems to be extraordinarily suitable for prolonged maintenance of human and monkey cells. We have prepared our medium essentially according to the methods described by Baron and Low.

'Instant Pet Nonfat Dry Milk' (manufactured by Pet Milk Co., St. Louis, Mo.), is reconstituted according to the directions of the manufacturer in triply distilled water. The milk is autoclaved at 7·5 lb. pressure for 15 min. The *p*H of the autoclaved milk is then brought to approximately 7·2 by the addition of 5 c.c. of 5 per cent sodium bicarbonate per 100 c.c. of reconstituted milk. The autoclaved milk is then added to medium No. 199 to prepare a mixture containing 20 per cent fatless milk and 80 per cent No. 199. Prior to use, the *p*H of the 20 per cent fatless milk medium is adjusted to approximately 7·4 with 5 per cent sodium bicarbonate. Medium No. 199 is prepared from a stock concentrate obtained from Microbiological Associates, Bethesda, Md. We add 50 units of penicillin to each ml. of medium No. 199.

Seven tube-cultures of *P*388 cells grown without plasma-clot in a medium of 40 per cent human serum and 60 per cent No. 199 were washed twice in serum-free No. 199 fluid and placed in the medium consisting of 20 per cent fatless milk and 80 per cent No. 199. They were then infected with *Mycobacterium lepraemurium*. Examination of one of these cultures after Ziehl–Nielsen staining showed large numbers of intracellular acid-fast bacilli. During the first week after infection, the *p*H of the media in the tubes was lowered rapidly and daily feeding was necessary. During the second week, many of the cells developed nuclear pyknosis and began to detach themselves from the glass surface. After three weeks, very few cells still adhered to the dependent surfaces of the tubes, and *p*H changes in the media were extremely slow. Seven weeks after transfer to the milk medium, small clusters of proliferating *P*388 cells were seen in several of the tubes. These slowly increased in number and by fourteen weeks the tubes had become repopulated with cells morphologically indistinguishable from the original *P*388 cultures. Acid-fast bacilli were not seen in these cells. At this time, daily feeding of cultures became necessary.

Cells from a single tube were scraped from the glass surface with a pipette, resuspended in fresh fatless milk medium, and transferred to a 2-oz. prescription bottle. They rapidly became adherent to the glass surface of the bottle and proliferated. After one week, they covered the dependent surface of the bottle. Transfer of cells from other tubes to bottles was easily accomplished in a similar fashion and repeated subcultures have been possible. Cells grown in bottles have been transferred to cover-slips in Leighton tubes for cytological studies with no difficulty. It has been shown that the milk-adapted cell line is still actively phagocytic and studies with *M. lepraemurium* in these cells are in progress.

It has thus been possible to adapt phagocytic tissue-culture cells from a murine malignant lymphoma to growth in a serum-free medium containing 20 per cent autoclaved fatless milk. The use of such cells, free of serum antibodies and inhibitors, in studies of intracellular bacteria as well as rickettsia and viruses is suggested.

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The Dendritic Cell System and Mast Cells in Non-Epidermal Stratified Squamous Epithelium

The occurrence of the dendritic cell system has been investigated in mammalian non-epidermal stratified squamous epithelium. Epithelium was split from the underlying connective tissue by treatment at 4° C. overnight[1] with pancreatin in Hanks balanced salt solution[2] adjusted to *p*H 7·8 with sodium bicarbonate. The epithelium was stained supravitally in a solution of 0·01 per cent toluidin blue and 0·05 per cent sodium bicarbonate in Hanks balanced salt solution for one hour or longer[3] at

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