following oral administration of glycerine extracts of adrenal cortex to human patients. Recent work on experimental hypertension indicates that the cortical hormone is concerned with blood pressure.[4, 5]

The observation that cortical hormone has a direct effect upon blood pressure of adrenalectomized dogs which is separable and distinct from its functional control of the internal fluid and electrolyte balance of the body, in no way invalidates the earlier conclusions based upon the hormone's "salt-water" action, as offering a rational explanation of many of the phenomena occurring in adrenal insufficiency. The 2 effects of the hormone when considered together, adequately explain the circulatory collapse of adrenal insufficiency.

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Agar Slant Tissue Cultures of Typhus Rickettsiæ (Both Types).

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For some time the writers have been engaged in efforts to obtain typhus fever Rickettsiæ cultures on a scale sufficiently large to permit vaccine-production. While the X-rayed-rat technic, elsewhere published by one of the writers with Castaneda,[1] is entirely adequate for vaccine-production with the murine strains, it has consistently failed with the classical European type. Tissue-culture vaccine-production would undoubtedly be the most practical method of producing vaccines on a large scale against the European disease, and the effectiveness for immunization of formalin-killed tissue-culture organisms has already been demonstrated, both by Kligler and Aschner[2] and by one of the writers with Macchiavello.[3]

Work done on the physiology of Maitland cultures by one of the writers with Schoenbach[4] indicated that whereas virus agents mul-

4 Goldblatt, H., *Ann. Int. Med.*, 1937, **11**, 69.

5 Jeffers, W. A., Lindauer, M. A., and Lukens, F. D. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 260.

1 Zinsser, H., and Castaneda, M. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

2 Kligler, I. J., and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

3 Zinsser, H., and Macchiavello, A., *J. Exp. Med.*, 1936, **64**, 673.

4 Zinsser, H., and Schoenbach, E. B., *J. Exp. Med.*, 1937, **66**, 207.

tiply in such cultures only during the period of active tissue metabolism—the Rickettsiæ begin to multiply most profusely after metabolism has slowed down or ceased.

This encouraged the hope that it might be possible to develop a method of Rickettsia multiplication in which larger amounts of tissue—and consequently a larger yield of the organisms—could be used. The problem was to find a technic in which the tissue could be held to a minimum of metabolic activity under circumstances of controlled pH and retardation of autolytic change.

Along these lines of effort we have been able to obtain large yields of Rickettsiæ of both the murine and classical (European) types by the following method:

A 4% agar solution in water is mixed with an equal volume of double strength Tyrode's solution to which 50% horse serum has been added before filtration. Phenol red is added in order that the reaction of the tubes may be observed. The initial reaction should be approximately pH 7.4 to 7.6.

Guinea pig tunica is minced, as for Maitland culture, and is heavily inoculated by dropping the centrifugalized concentrate of a preceding culture on the tissue in a Petri dish. After 5 or 10 minutes at room temperature, this tissue is then "buttered" on the surface of the agar, and the tubes are closed with a rubber stopper through which there has been passed a glass tube drawn to a capillary, as shown in Fig. 1. The purpose of the glass tube is that by either breaking or sealing the capillary and allowing any formed carbon dioxide to escape or to accumulate, the reaction can be to some extent controlled, if that becomes necessary. The most important single point in the technic essential to success is reaction-adjustment, and it seems that in most cases, if the initial reaction is as stated, adjustment by the capillary is not necessary in the course of the 8 or 10 days necessary for development of the cultures.

FIG. 1.

At the end of 6 days, multiplication is already apparent and after 10 days smears from the tissue on the surface of the agar show growth of Rickettsiæ. The organisms not only crowd many of the cells which are reasonably preserved, but considerable numbers of scattered Rickettsiæ are found throughout the tissue detritus.

Whether or not these actually grow in the diffused cell material or represent the yield of ruptured cells has not yet been determined. At any rate, a single culture of this kind gives a better total yield than three or four of the larger Maitland flasks.

It is still to be determined why large amounts of tissue on slanted agar do not inhibit Rickettsial growth in the same way in which even a slight excess of tissue prevents multiplication in liquid cultures, even when reaction-changes are controlled. It is probable that since active metabolism seems to delay rather than favor Rickettsial multiplication, the cells laid on the surface of agar may, from the beginning, exhibit a very low level of metabolic exchange. In consequence, changes of reaction and the possible production of inhibiting substances take place to a slight degree and slowly, and the products diffuse away from the cells into the agar.

Since heavy inoculation is necessary in order to obtain yields suitable for vaccine production—which is the ultimate purpose of the work—we have had to carry these cultures to the 5th agar generation and a dilution, in terms of tissue used—of the original Maitland culture of approximately 1 to 700—before we could be sure we were not dealing with organisms carried over.

We are at present engaged in improving methods of controlling the reaction and determining the suitability of embryonic tissue and other cellular material more easily procurable in considerable quantities than guinea pig tunica.

Although probably not in its final form, the method appears to us to offer hope of solving in a simple manner the problem of vaccine production for human protection against both the murine and the classical European varieties of typhus fever. It is not out of question that it may be applicable to virus cultivation as well since the cells are no doubt to some extent active for 48 hours.