the untreated controls (Group III) ($P < 0.01$); the enlarged, hypertensive heart had not regressed significantly.

Discussion. Testosterone treatment accompanied with ovariectomy not only reversed an established hypertension of NAE-female rats but also appeared to cause a regression of adrenal mass (Group IV), since, at the time treatment was initiated, the adrenals of Groups III and IV were presumably equal and close to maximal size. It has been reported(5) that testosterone provoked adrenal atrophy when administered to female rats under other experimental conditions. However, reduction of endogenous testosterone by castration of NAE-male rats did not produce high blood pressure even though all NAE-males showed some adrenal regeneration. It is possible this phenomenon is dependent upon the presence of an adequate concentration of estrogens. Estrogens have been reported to produce adrenal hypertrophy (6) and hypertension in rats(7,8). The response of the castrated NAE-male rat to exogenous estrogen is being investigated.

Summary. Evidence has been presented that NAE-female rats but not NAE-males demonstrated adrenal regeneration accompanied with hypertension. Testosterone administered to NAE-female rats inhibited regeneration of the enucleated adrenal and development of hypertension. Testosterone caused a reduction in the blood pressure of ovariectomized, hypertensive NAE-female rats. Castration was not effective in promoting adrenal regeneration or hypertension in the male rat.

The authors wish to thank the following members of The Upjohn Co.: Mr. S. C. Lyster for performing the ovariectomies. Dr. H. D. Webster for histological studies and Mr. F. LaPlante for care of the animals.

1. Skelton, F. R., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 342.
2. Skelton, F. R., and Guillebeau, J., *Endocrinol.*, 1956, v59, 201.
3. Skelton, F. R., *Am. J. Path.*, 1956, v32, 1037.
4. Stafford, R. O., Thole, L. C., and Olson, K. J., *Endocrinol.*, 1953, v52, 292.
5. Pincus, G., and Thinman, K. V., *The Hormones*, Academic Press, New York, 1950, v2, p50.
6. Tepperman, J., Engel, F. L., and Long, C. N. H, *Endocrinol.*, 1943, v32, 373.
7. Grollman, A., Harrison, T. R., and Williams, J. R., *J. Pharm. Exp. Ther.*, 1940, v69, 149.
8. Leathem, J. H., and Drill, V. A., *Am. J. Physiol.*, 1943, v139, 17.

Received April 5, 1957. P.S.E.B.M., 1957, v95.

A Method of Isolating Normal and Fowlpox Infected Chorioallantoic Epithelium.* (23175)

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In this laboratory we are concerned with an investigation of certain chemical changes which appear to be induced in chorioallantoic epithelium infected with fowlpox virus. Goodpasture(1) has shown that in fowlpox infection the hyperplastic ectoderm contains numerous large inclusions, that to a lesser degree the entoderm is involved as well, and that there is accompanying thickening of the mesoderm. It would be very desirable to obtain infected epithelium in quantity without inclusion of mesodermal elements which may tend to obscure the results.

Procedure. Three different batches each consisting of 3-6 dozen embryonated hen eggs were inoculated with fowlpox on the chorioallantoic membranes by the Goodpasture method(2) and incubated for 5 days at 37°C. A comparable number of uninoculated control eggs were incubated under the same conditions. Infected and control membranes were collected aseptically in cold physiological saline and refrigerated for several hours until

* Supported in part by U.S.P.H.S. Grant.

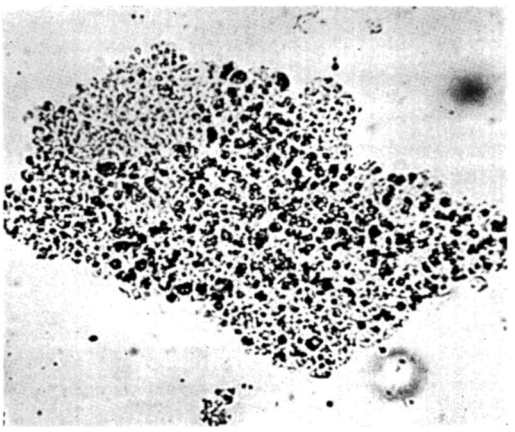

FIG. 1. Unstained wet mount of epithelium of infected chorioallantoic membrane. The dark masses represent intracytoplasmic inclusions. The tissue was teased flat with some tearing, freeing individual inclusions and minute fragments. 120 ×.

ready for use. Ten to 15 membranes at a time were shaken by hand in 10 ml of cold saline with glass beads in a 125 ml rubber stoppered flask for approximately 30-120 seconds. Preliminary experiments indicated that 30 to 60 seconds were adequate for infected tissue whereas normal tissue required longer agitation. The suspension was decanted from the membranes and like preparations were pooled and further diluted with cold saline and allowed to sediment by gravity for 30 minutes to 1 hour. Small fragments rapidly settled out, leaving red cells and debris in suspension. This was aspirated and fresh saline added. This procedure was repeated and the tissue washed 8 to 10 times until the supernatant was crystal clear. Small aliquots were withdrawn for microscopic examination and the remainder centrifuged for 10 minutes at 10,000 rpm in a Servall Angle Centrifuge. The resulting firm pale grey bottom material was frozen and lyophilized. Representative samples of the shaken membranes were fixed in formalin for subsequent histological examination. Several normal and infected membranes were included for comparison and are referred to as control tissue.

Results. Microscopic examination of the clean sedimented material from the infected membranes revealed numerous epithelial fragments varying in size from 0.1 to 2 mm. Red cells were rare and no mesoderm was identified. After study of the fixed and stained control membranes several generalizations as to origin of the epithelium appear justified and are consistent with recorded data(1). Many of the fragments were composed of cells containing typical intracytoplasmic inclusions, characteristic of fowlpox (Fig. 1), and appeared ectodermal in origin. Other pieces of tissue, though hyperplastic, contained few or no inclusions and were probably derived from the entodermal layer. Study of hemotoxylin and eosin stained shaken membranes showed the entoderm to be stripped free in many instances, but present in others, whereas the ectodermal layer was uniformly denuded.

Examination of sediment prepared from normal membranes revealed numerous small fragments of epithelium of mixed ectodermal and entodermal origin, and hemotoxylin and eosin slides of the parent membranes showed considerable desquamation of both cell layers.

Comparison of the lyophilized materials from infected and normal sources consistently revealed that a bulkier and heavier pellet was obtained from the infected membranes. For example, the epithelium obtained from 72 infected eggs weighed 366.7 mg in contrast to 77.9 mg derived from 42 normal eggs. The yield is understandably quite variable since the primary purpose is purity of the preparation.

Discussion. Putnam(3) has pointed out the difficulties attendant on determining chemical changes in tissues infected by viruses because of inflammation and cellular degradation. Our results have shown that the infected tissue in which the specific lesions are numerous can be separated from the mesoderm. This separation is clearly an advantage when chemical determinations are contemplated. The technic described possibly has other uses, as preliminary experiments have shown that the small epithelial clumps are suitable for tissue culture.

Summary. A method is described for isolating chorioallantoic epithelium from normal and fowlpox infected embryonated hen eggs.

1. Woodruff, A. M., and Goodpasture, E. W.,

Am. J. Path., 1931, v7, 209.

2. Buddingh, G. J., in *Rivers Viral and Rickettsial Infections of Man*, 2nd Edit., J. B. Lippincott Co., 1950, 109.

3. Putnam, F. W., *Ann. Rev. Biochem.*, 1956, v25, 147.

Received April 5, 1957. P.S.E.B.M., 1957, v95.

Effect of Acute Liver Damage Plus Hypoxia on Plasma Erythropoietin Content. (23176)

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The factors which regulate erythropoiesis are gradually being elucidated. That a circulating humoral factor in the plasma is important in stimulating red cell production under many circumstances is gaining increasing acceptance(1-6). This material has been demonstrated in the plasma from experimental animals and humans with anemias of several different etiologies(7,8), and from several animal species(9-11). A consistent finding has been that the plasma from animals made anemic with phenylhydrazine was more active in stimulating erythropoiesis than the plasma from bled animals(12-14). Some have maintained that degree of anemia was the important factor here, a more severe anemia being produced by phenylhydrazine administration than could be produced by bleeding, with survival of the animal. Recently the observation has been made that this enhanced erythropoietic activity of plasma from phenylhydrazine-anemic animals is in some way related to liver damage(15).

The present study is a further effort to clarify the status of the liver as a controlling influence on the presence and titer of erythropoietic factor in the plasma. During the past year and one-half efforts have been made to demonstrate EPF* in the plasma of normal rats placed in chambers and subjected to atmospheres of 8-12% oxygen for varying periods of time. These results were uniformly negative. At no time were we able to demonstrate any ability of the plasma from these rats kept in this low oxygen atmosphere to stimulate increased Fe^{59} uptake in recipient hypophysectomized or normal rats. It was postulated that perhaps if acute liver damage were induced prior to placing the animals in the chamber an erythropoietic factor might then be demonstrable in their plasma. This proved to be the case and the results are here reported.

Materials and methods. The chambers were constructed of plexiglass. Into each chamber was directed a mixture of nitrogen and oxygen, the proportions of which, along with ambient pressure, determined the simulated altitude. The flow of nitrogen and oxygen was regulated by a rotameter type of flowmeter† and remained quite constant over the experimental period. The chamber O_2 concentration was checked by reading of the oxygen and nitrogen flowmeters and also by a portable Beckman Oximeter. Random air samples were also taken from the chamber in a Douglous bag and checked with a mass spectrograph for O_2, N_2 and CO_2. Good agreement in results was obtained. A flow of approximately 5 liters/minute was maintained through the chamber and soda lime and sulfuric acid were placed to minimize CO_2 and humidity levels.

Ten to 15 normal rats were placed in each chamber and kept there for periods ranging from 48 hours to 5 days. Food (Purina Laboratory Chow) and water were replaced as necessary. At the end of the experimental period the rats were removed, bled from the dorsal aorta and the plasma frozen and stored. A final hematocrit was done on each animal and no animal with

* Refers to plasma erythropoietic factor.

† Chicago Anesthesia Equipment Co.