Immune rabbit sera were prepared with the St. Louis Broun 1933 strain and the Japanese Nakayama strain.

Neutralization tests shown in Table II indicate that St. Louis No. 3 virus was neutralized equally well by homologous and St. Louis Broun 1933 immune sera (10 to 100 M.L.D.), but that the Broun strain was less effectively neutralized in the St. Louis No. 3 (1 to 10 M.L.D.) than in the homologous immune sera (10 to 100 M.L.D.). Thus there appeared slight immunological differences between the 2 St. Louis strains.

Table III shows that (1) St. Louis No. 3 virus was not neutralized by the Japanese Nakayama immune serum and (2) the 5 Japanese strains were neutralized either not at all or only in the M.L.D. dilution by St. Louis No. 3 immune serum and human St. Louis convalescent sera.

Mice immunized with St. Louis No. 3 virus were not immune to as little as 1 M.L.D. of the Nakayama virus.

Thus, on present evidence, we regard the relationship between St. Louis and Japanese B virus to be as stated in 1938.[2] They are closely related with respect to the epidemiology of the diseases which they produce. Together with louping-ill and equine encephalomyelitic viruses, they possess similar filterability-characteristics with Elford membranes. In animal species, St. Louis, Japanese, and louping-ill viruses show general similarity and Japanese B and louping-ill viruses appear identical. And finally, St. Louis, Japanese, and louping-ill viruses, in spite of an occasional cross-neutralization not exceeding 1 to 10 M.L.D., remain immunologically readily distinguishable.

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Androgenic Assay of the Human Fetal Adrenal.*

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It is indicated from the study of castrate men and women that there is an extragonadal source of sex hormones of some abundance. This was suspected to be the adrenal long before the actual demonstration by Reichstein[1] that small amounts of androgenic material

* The autopsy material for this study was collected at the Baltimore City Hospitals and the Johns Hopkins Hospital, Baltimore.

1 Reichstein, T., *Helv. Chim. Acta*, 1936, **19**, 223.

could be chemically isolated from beef adrenals. The strongest reason for suspecting the adrenal as a source of these hormones has been the occasional occurrence of sexual changes (the "adrenogenital" syndrome)[2] and the urinary excretion of large excesses of some of these hormones[3, 4, 5] in individuals harboring a neoplasm of the adrenal gland (and the regression of the sexual changes and diminution of the urinary hormone excretion following surgical removal of the tumor in some of these cases). Marked bilateral hyperplasia of the adrenal cortex is associated with other cases of virilism in women and is the almost invariable accompaniment of congenital female pseudohermaphroditism.[6] Furthermore, androgenic hormone has been found in the adrenal tissue itself in a case of macrogenitosomia praecox[7] with excessive hyperplasia of the adrenals. The majority of these observations, therefore, suggest androgenic more than estrogenic secretion by the adrenal. Nevertheless, adrenal cortical tumors and some degree of cortical hyperplasia are common and only a very few of these cases have associated sexual changes. Grollman[2] has made an attractive suggestion that this is due to the existence of 2 distinct functional types of cell in the human adrenal cortex, the one concerned with producing a vital hormone and the other with producing an androgenic hormone. The latter function he believed to reside in the great bulk of cortical tissue composing most of the gland in fetal life which promptly and regularly involutes after birth.[8] The persistence of a few cells of this fetal tissue has been postulated in the juxta-medullary zone of the gland even into adult life[9] and it is from these cells that Grollman has suggested that those tumors and hyperplasias influencing the sexual characters arise. There is some support for such a hypothesis on morphological grounds[9, 10] although it is admitted that no rigid histological criteria are available for establishing it.

It was with the hope of adding some evidence on this hypothesis that adrenal tissue was collected from human fetuses for biological

2 Grollman, A., *The Adrenals*, Williams and Wilkins Co., Baltimore, 1936.

3 Frank, R. T., *J. A. M. A.*, 1937, **109**, 1121.

4 Koch, F. C., *Ann. Int. Med.*, 1937, **11**, 297.

5 Friedgood, H. B., and Whidden, H. L., *New England J. Med.*, 1939, **220**, 736.

6 Young, H. H., *Genital Abnormalities, Hermaphroditism, and Related Adrenal Diseases*, Williams and Wilkins Co., Baltimore, 1937.

7 Wilkins, L., Fleischmann, W., and Howard, J. E., *Endocrinology*, 1940, **26**, 385.

8 Elliott, T. R., and Armour, G., *J. Path. and Bact.*, 1911, **15**, 481.

9 Goormatigh, N., *Le cortex surrénal humain dans les plaies de l'abdomen et aux périodes intéressantes de la vie sexuelle*, Liege, 1922.

10 Broster, L. R., and Vines, H. W. C., *The Adrenal Cortex*, London, 1933.

assay. The material was obtained from 20 stillborn and newborn† fetuses autopsied at from 4 to 44 hours postmortem. The fetuses varied from 18 to 40 weeks in period of gestation. Approximately 100 g of wet adrenal tissue was obtained. The glands were chopped up and preserved in neutral acetone in tightly covered bottles in the cold. The preliminary extraction was accomplished by 2 changes of this acetone, 10 and 5 fold excesses respectively, and 2 additional excess quantities of ethyl ether for 24 and 48 hours respectively. The acetone fractions were combined and the acetone removed at less than 40°C under reduced pressure. The residue from this was taken up in ether and all the ether fractions combined and dried with solid anhydrous sodium sulphate. About 40% of the ether extract was condensed into a small convenient volume of olive oil by evaporation on the steam bath-(A). This method is the same as that employed by Parker and Tenney[11] in their study of the estrogenic content of the tissues in pregnancy except that the additional precaution of vacuum distillation was used in removing the acetone in order to keep the temperature under 40°C.

The remaining 60% of the ethereal solution was extracted with 5 small portions of 10% aqueous NaOH to remove the estrogenic substances, then washed with several changes of distilled water, dried again with anhydrous sodium sulphate and condensed into a convenient volume of olive oil on the steam bath-(B).

The method of assay for androgenic activity was a slight modification of the chick comb test[12] recommended to me by Dr. Ralph B. Oesting. The oily solution was directly applied to the comb with a pipette. Day-old chicks were used. A solution of testosterone propionate in oil was the standard of comparison. Estrogenic assays were done by the immature rat uterus method.[13] Crystalline estrone in oil was the standard of comparison.

Fraction A was first assayed for both androgen and estrogen. No androgen was detected but the estrogen content was estimated as equivalent to 272 γ of estrone per kilogram of fresh tissue (2720 I.U. estrogen per kg). Fraction B was therefore prepared in order to have an androgen fraction free of estrogen since it is known that

† To avoid including involuted fetal cortex, no glands were used from babies living more than 48 hours after delivery. Obviously autolyzed specimens were not included.

11 Parker, F., Jr., and Tenney, B., Jr., *Endocrinology*, 1938, **23**, 492.

12 *a.* Dorfman, R. I., and Greulich, W. W., *Yale J. Biol. and Med.*, 1937, **10**, 79; *b.* Frank, R. T., Klempner, E., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 853.

13 Lauson, H. D., Heller, C. G., Golden, J. B., and Sevringhaus, E. L., *Endocrinology*, 1939, **24**, 35.

estrogen may inhibit the comb response in some degree. The androgenic assay on Fraction B was likewise negative in such quantity as to indicate a concentration less than equivalent to 1 mg testosterone propionate per kilo of fresh tissue. This indicates an androgenic content (if any) of less than 10 I.U. of activity per kilo of fresh adrenal.‡

The significance of these observations is admittedly limited. A positive result would have permitted more positive conclusions. All that can be said is that no support has been found for the hypothesis that the human fetal adrenal produces androgenic hormone. The technic of extraction and assay is well standardized and controlled. Some degree of autolysis of the tissue before extraction was inevitable but previous experience indicates that this would be no detriment.[11] It would be vain to conjecture what concentration of hormone should be expected in a tissue functionally androgenic. The hormone content of the endocrine glands is known to vary widely under different physiological circumstances. The hyperplastic adrenal in the case of macrogenitosomia praecox mentioned above[7] contained an estimated 715 I.U. of androgen per kilo of fresh tissue.

The estrogen concentration found was similar to that found in the fetal adrenal by Parker and Tenney[11] in their study of the estrogen content of several maternal and fetal tissues in pregnancy. As these authors have demonstrated, this cannot be taken as evidence of the site of formation of estrogenic hormone in this instance, since in pregnancy all the tissues of both mother and fetus contain large amounts of the hormone. The demonstration of estrogen in this tissue, however, does afford some control of the efficacy of the extraction.

Summary. In spite of some evidence suggesting an androgenic function of the human fetal adrenal cortex, it has not been possible to demonstrate any androgenic activity by biological assay of the tissue by a method adequate to detect as little as 10 I.U. per kilo of fresh tissue.

‡ Repeated comparisons have shown that by this method of assay testosterone propionate has an approximately equal activity to the same weight of cis-androsterone, the international standard.