activity was found. The fraction insoluble at pH 2.5 contained both follicle-stimulating and luteinizing activities. 3. The results are discussed from the viewpoint that follicle-stimulating hormone may exist in pituitary tissue in different forms.

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Failure to Demonstrate Skin Groups Analogous to Blood Groups Using Human Skin Cultures.* (20893)

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The question as to whether human skin partakes of the group characteristics of blood is still unsettled. The lack of definitive work on this problem is due in part to the fact that skin cells being fixed cells do not lend themselves to the typing procedures applicable to erythrocytes. In the present work the tissue culture method was utilized in a study of this problem.

Materials and methods. Specimens of human skin from donors of known blood types were obtained at the operating table, cut in varying thicknesses with the electric dermatome or in full thickness with the ordinary scalpel. The specimens were immediately placed in a mixture of 90% Tyrode's solution and 10% pooled human serum and refrigerated until explants were made in from one to two hours. The Maximow double coverslip method in which the explant was embedded in a plasma clot after the method of Burrows(1), was used. Under these conditions explants showed growth in from 3-4 days and reached maximum growth after 6-12 days, and after 15-17 days began to regress and were discarded. The following groups of experiments were performed: A. 115 cultures (from 29 donors) of skin grown with pooled human cord serum as nutrient and at their maximum growth, were first washed with Tyrode's solution and then treated as follows: 1. 82 cultures from blood Group A donors with one drop of Anti A serum. 2. 33 cultures from blood Group B donors with one drop of Anti B Serum. The sera were commercial "high potency antisera from donors whose antiserum titer had been stimulated by either A or B specific substances for a suitable period previous to their blood donation,"(2) and whose agglutinative power against the corresponding erythrocytes had been checked in this laboratory before they were used. B. 98 skin explants from donors whose blood groups had been previously determined (62 A and 36 B) were made according to the method described above, but with one drop of the corresponding antisera as nutrient from the start. C. A third group of experiments was suggested by the work of Bassett, Earle and Campbell(3) which in turn were suggested by an observation made by Farr and his co-investigators(4). Farr and his co-workers found that in the plasma and serum of rabbits there is "normally present a protective factor against the leukopenic effect of total body irradiation but that this factor is largely destroyed by total body or *in vitro* irradiation with 800r. They have also found that there is a naturally occurring protective factor against pyrogens in the blood of rabbits but the relationship between the irradiation factor and the pyrogen factor has not been studied as yet." Bassett, Earle and Campbell observing that the serum of rabbits previously inoculated with human skin material had no effect on cultures of human skin, suspected that a protective factor might likewise be

* Based on work performed under contract with the Atomic Energy Commission.

present in the rabbit serum, inhibiting a cytotoxic effect. They showed that after irradiation or chemical purification the serum then became cytotoxic for skin cultures. Since irradiated or purified serum of the control unsensitized rabbits did not exert this cytotoxic effect the possibility of an artifact resulting from irradiation or purification was believed to have been ruled out.

This work raised the possibility that a similar protective substance is present in the normal serum which while it does not inhibit the effect on incompatible erythrocytes may still be inhibitory against adverse effects on skin, should these effects be found. For purposes of this last group of experiments samples of Anti A and Anti B serum were fractionated by Dr. Dan Campbell into two portions, one containing Alpha 1 and Alpha 2 globulins and the other gamma globulins. Preliminary tests showed that the agglutinins resided in the first fraction. In order to eliminate the possibility of any protective factor being carried over in the plasma clot, perforated cellophane of suitable diameters was used in place of the embedding clot. 12 skin cultures from blood Group A donors thus grown were treated with one drop each of both fractions of Anti A and 12 skin cultures from blood Group B donors were treated with one drop of the corresponding fractions of Anti B serum.

The following criteria were sought in all 3 groups of experiments: (1) Signs of clumping or agglutination as found when erythrocytes are treated with an incompatible serum. (2) Lysis. (3) Other gross morphological changes. (4) Inhibition of growth. All the cultures in Group A were under microscopic observation for 2 hours after treatment with the antisera, then reincubated for 5 days and again observed. All the cultures in Groups B and C were observed daily from 48 hours after explantation.

Results. None of the cultures in the 3 groups of experiments, following treatment with antisera, showed any of the 4 criteria set forth above. On the contrary growth continued at apparently the same rate and for the same duration after application of the incompatible sera or fractions of sera as in cultures treated with the pooled nutrient serum. In some experiments the treated cultures showed more luxuriant growths than the corresponding controls.

Summary. Skin cultures from human donors of blood Groups A and B after treatment with respective antisera showed no detectable morphologic changes under the microscope. Skin explants from donors of blood groups A or B, grown with their respective antisera as nutrients likwise showed no detectable morphologic differences from control cultures.

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