inoculation and the absence of any signs of damage to the host tissues at the site of inoculation are, however, some indications that this is not the correct explanation.

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HUMAN SERUM, AGE AND MULTIPLICATION OF HOMOLOGOUS FIBROBLASTS[1]

THE experiments of du Noüy[2] have shown that the rate of cicatrization of a sterile wound is a function not only of the area of the wound but also of the age of the patient. Simply stated, the area of the wounds being the same, the rate of repair is faster in young individuals than in old. Later work by Carrel and Ebeling,[3] and by Baker and Carrel,[4] has demonstrated that these findings may be explained on the basis of progressive physico-chemical changes which take place in the blood plasma during the lifetime of the individual. These authors have shown that the inhibiting action of homogenic and heterogenic serum on the growth of pure cultures of chicken fibroblasts is directly proportional to the age of the animal from which the serum is taken. This process of aging, which advances more rapidly during early life, is due, in part at any rate, not only to the loss of growth-activating substances, but also to an increase in concentration of inhibiting proteins and lipoids, particularly the latter. The curve representing the increase of these substances resembles the curve of the index of cicatrization, of du Noüy's formula, as a function of the age of the individual. After extraction of the lipoids, the remaining proteins are found to be far less inhibiting than the whole serum. Also, when the serum of an old fowl is diluted so that its protein concentration corresponds practically to that of a young fowl, its growth-inhibiting power, although markedly decreased, is still higher than that of the serum from the younger animal. Carrel[5] has also shown that the toxic substances which characterize old age are being continuously secreted by the tissues. Their progressive accumulation in the blood is not the result of lack of elimination from the circulation; it is rather an expression of the condition of the tissues. When the blood plasma was removed from old dogs by plasmaphaeresis, and was replaced by Tyrode solution, their serum was less inhibiting for a short time thereafter, but rapidly regained its former characteristics as the plasma was regenerated by the tissues. This recovery took place too rapidly to allow of a definite modification of the tissue cells.

The experiments here to be reported bring additional evidence of a confirmatory nature by demonstrating a striking contrast between the action of infant and adult sera on colonies of human fibroblasts cultivated *in vitro*. The fibroblasts were derived from a subcutaneous rheumatic nodule removed at biopsy from a seven year old male, and have been cultivated for three months in flasks on a medium consisting of a mixture of 20 per cent. adult human serum and 80 per cent. chick embryo tissue juice. When the experiments were made, this strain had been cultivated for almost two months and had been subdivided and transferred seven times. The cultures selected for the experiments were divided and the two halves of each were placed in separate flasks. One half of each was treated regularly with serum obtained from a 14 months old infant and the other half with serum from a 27 year old adult. In order to intensify the effect of the sera to be compared, the usual proportions were reversed so that the mixture consisted of 80 per cent. serum and 20 per cent. chick embryo juice. The results obtained from three such experiments which were made simultaneously, and with the same materials, have been averaged and presented in Fig. 1. It may readily be seen that the cultures

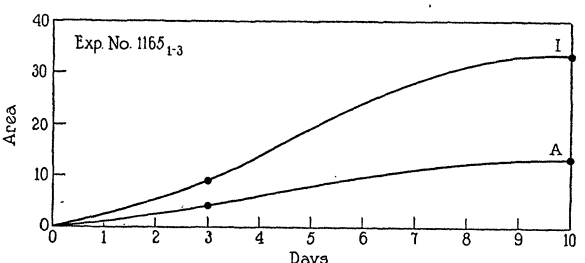

FIG. 1. Comparison of the rate of growth of human fibroblasts in the sera of a 14 months old infant and a 27 year old adult. I = infant's serum; A = adult serum.

treated with the infant's serum attained an area in ten days which was over 150 per cent. greater than that reached by those treated with adult serum.

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[1] From the Laboratories of The Rockefeller Institute for Medical Research.

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Research, for material used in connection with the experimental work.

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A PRACTICAL TEST FOR POTENCY OF EXTRACT OF CORPORA LUTEA

THE large amount of work which has been done, in the last few years, upon extracts of corpora lutea, and the results of that work, have urgently called for a suitable test for potency. Corner's and Allen's[1] studies on progestational proliferation in the rabbit, resulting from the physiological action of their extract of the corpus luteum, and Hisaw's and Leonard's[2] similar results, provided a useful test, but one which had several practical objections, arising primarily from the relatively large size of the test animal, and the consequent expense of obtaining and maintaining the animals and of making the tests.

To overcome these objections, W. M. Allen[3] attempted to transfer the application of this technique, using rats as test animals. As a result of thorough studies, he concluded that "The histological alterations of the rat's endometrium during pseudopregnancy are insufficient to warrant the use of these changes as a test for corpus-luteum extracts." At the same time we obtained wholly confirmatory, though as yet unpublished, results in this laboratory. We also had similar results in studies conducted on the uterus of mice.

The results of our studies upon histological changes in the vaginal mucosa of mice have, however, been very satisfactory, and we now present some of them as the basis of a practical test for potency of extracts of corpora lutea.

From histological studies made upon the vaginae of 19 normal mice in different stages of the oestrous cycle, and of pregnancy, it was apparent that the normal changes in the histological picture of the vagina of mice is similar to that described by Long and Evans[4] for the rat.

Histological changes of the vaginal mucosa, comparable to those occurring during pregnancy, were found when normal, adult, unmated, female mice were treated, just after oestrus, with an extract of corpora lutea, in all of 13 test animals, treated with daily injections of extracts for periods ranging from 3 to 14 days. For test purposes, however, it is recommended that periods of more than 7 days be used.

At present we recommend the following test: Beginning the day after oestrus, make daily injections of 0.25 cc. of corpus-luteum extract per test animal for a period of 9 to 14 days. The vagina, upon being preserved, sectioned and stained, according to the usual histological technique employed in such cases, will show, with an extract potent for maintaining pregnancy in ovariectomized, pregnant animals, a histological picture characteristic of pregnancy, *i.e.*, absence of cornified epithelium and of infiltration of leucocytes, presence of vacuolization of the superficial and middle cell layers, mucous transformation, *et cetera*.

This test has several obvious advantages. The test animals are small, easy and inexpensive to obtain and maintain, and the test occupies a relatively short period. Furthermore, 2 to 3 ccs. of extract is sufficient to make a test upon one animal. It may even be that a smaller dosage than that of 0.25 cc. per day will be found to be effective, as the minimal effective dose has not yet been ascertained. Experienced workers will also probably find that the period of time recommended for the test may be reduced with safety.

The extract used in these tests was one prepared by Dr. J. J. Pfiffner, and has been the basis of much of our work upon the physiology of corpus-luteum extract. It is similar to Corner and Allen's extract, save that methyl alcohol is used in place of ethyl alcohol as the first extracting agent.

Similar tests made as controls upon five animals with Swingle and Pfiffner's[5] extract of the adrenal cortex did not bring about a pregnant appearance of the vaginal mucosa in non-pregnant mice.

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