ognized as a feature of the genotype and as being ostensibly unaffected by environmental considerations. The fact that we find individuals with weakness of the masculine component most heavily represented in the smoking group, and especially in the heavier smoking category, suggests that for a specified type of individual smoking may be a reflection of certain personality and behavioral traits which are characteristic of his biological make-up.

In this connection, it is to be noted that in a previous study the individuals with weakness of the masculine component "exhibit a characteristic pattern of traits which form a consistent and harmonious picture" (*4*). These less masculine persons tend to have an aversion for strenuous exercise and sports, are apt to be low in physical fitness for hard muscular work, and are often poor in muscular coordination. In the sphere of personality structure, they appear to be more sensitive in affect and manifest a greater degree of instability of the autonomic nervous functions. They are apt to be less well integrated and more ideational, creative, and intuitive. They are more frequently shy and asocial and more frequently have traits of self-consciousness and inhibition. In the formal intellectual functions they tend to rank higher in the verbal functions and possibly lower in the mathematical or number functions. Academically, they most often select the area of arts, letters, and philosophy as a college major, and their choice of career tends to follow these same lines of interest. What is significant here is the fact that this constellation of personality and behavioral traits for the individuals with weakness of the masculine component is for the most part not inconsistent with the findings of Heath (*2*) in his study of the differences between smokers and nonsmokers.

If further studies confirm the findings of this report, an important line of investigation should be explored which may bear on the question of the association of smoking with lung cancer and coronary heart disease. In view of the fact that smoking is found here to be significantly more frequent in individuals with weakness of the masculine component, then it would be pertinent to determine the differential frequency of lung cancer and coronary disease in males according to the strength of the masculine component in both smokers and nonsmokers. Such data would help establish whether differences exist in disease incidence between the classes of individuals within this genotypical body-build complex, and whether the element of smoking materially changes this incidence. Thus, it may be possible to secure evidence on the extent to which smokers and nonsmokers differ in their susceptibility to disease because of their biological nature, apart from the element of smoking itself (*8*).

CARL C. SELTZER
*Peabody Museum, Harvard University, Cambridge, Massachusetts*

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7. The statistical significance from which *P* values given in this report are derived is based on the chi-square method of computation.
8. This study was supported by the Tobacco Industry Research Committee and based on data of the Study of Adult Development of the Harvard University Health Service.

13 August 1959

## Method for the Study of Antigenicity of Homologous Whole Spleen Cells in Mice

*Abstract.* A method for the quantitative assay of the antigenicity of homologous mouse whole spleen cells in the system of A/Heston (donor) to C57BL/6 (recipient) is described. The assay is based upon the graded response of skin homografts to the numbers of donor cells injected intraperitoneally into the recipient prior to the skin graft. The response is linear with respect to the logarithm (base 10) of the number of cells injected.

Billingham and Medawar and their coworkers (*1*, *2*) have studied the sequence of changes in the skin graft implanted upon a homologous host. After an initial period of acceptance, there is a gradual rejection of the graft. If a second graft from the same donor, or from a donor of the same inbred strain, is implanted on the recipient, there is an accelerated rejection of the homograft. This phenomenon, as it has been defined in the experimental animal, is considered to have an immunologic basis. Accelerated rejection of a skin graft also occurs if a parenteral injection of whole splenic cells from the donor or donor strain is substituted for the initial skin graft (*2*). Billingham, Brent, and Mitchison (*3*) noted that, as the number of injected whole splenic cells from the donor strain of inbred mice was decreased (a decrease which represented a decrease in antigenic stimulus), then there was amelioration of accelerated rejection.

This observation suggested to us that there might be a dose-response relationship which could be defined by quantitative studies. We have found a direct correlation between changes in skin homografts and the number of splenic cells administered intraperitoneally before the application of the skin graft. The correlation has a sufficiently high degree of statistical significance to make the procedure described below of use in determining the antigenic potency of whole cells and cellular fractions.

1. *Experimental animals.* Pure-strain adult A/Heston (A/He) male mice (donors) and adult C57BL/6 male mice (recipients) obtained from the Roscoe B. Jackson Laboratories, Bar Harbor, Me., were used in these experiments.

2. *Preparation of cells.* Whole spleens (of A/He mice), freed from fat, were cut longitudinally, and the contents were gently pressed and washed with Ringer-Locke solution through a stainless-steel screen (40 to 60 mesh). Cell clumps were very gently dispersed into a uniform suspension by allowing the loose plunger of an all-glass homogenizer [described by Dounce (*4*)] to fall under its own weight with restraint. Cell counts were made in a hemocytometer, 3-percent acetic acid being used as the diluent. The first suspension was subdiluted (with Ringer-Locke solution) to the desired concentrations, and each of these was counted.

3. *Injection schedule.* Six groups of six mice each were injected intraperitoneally with whole homologous spleen cells as described above; a seventh, uninjected, group served as a negative control. The six doses were 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 $\times$ $10^6$ cells, respectively. The cell suspensions were so diluted that all injected volumes were 1.0 ml.

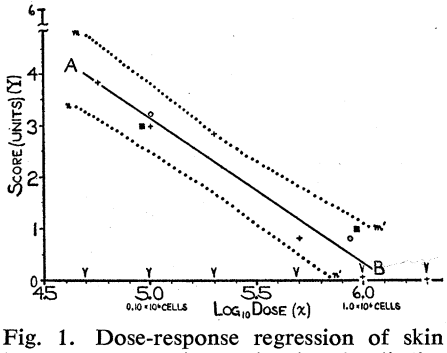


Fig. 1. Dose-response regression of skin homografts to prior antigenic stimuli (intraperitoneally-injected homologous spleen cells, isologous with graft). Experimental points for the regression line, + (in two subsequent experiments, ■ and ○); calculated regression line, *AB;* confidence limits at $P = 0.05$ for *AB*, *mm'*, and *nn'*. The equation for the regression is $Y = 17.18 - 2.81x$.

4. *Grafting.* Four days after injection, skin homografts (A/He), 1 cm square and of full thickness, were placed on the panniculus carnosus of each recipient and control, according to a modification of the technique devised by Billingham and Medawar (*5*).

5. *Histologic evaluation and scoring.* The method of scoring depends upon the observation that an initial skin homograft on a mouse is largely viable on the 6th day after grafting, while, in contrast, a homograft on a mouse which has received a prior antigenic stimulus in the form of either a skin homograft or of whole homologous cells introduced intraperitoneally is partially or totally necrotic by the 6th day (*6*).

Each homograft with its bed was therefore excised on the 6th day and fixed in neutral, buffered Formalin. Grafts were cut transversely into thirds, embedded in paraffin, and stained with hematoxylin and eosin. Sections were then examined microscopically. The percentage of necrotic epithelial cells of the surface and of the hair follicles of each homograft was estimated from the appearance of all three graft sections, and the results were scored with the following values: 0, 10, 25, 50, 75, 90, and 100 percent of surviving epithelial cells. For convenience in statistical analysis, these values were transformed to integers 0 through 6, respectively.

We have found that the scores of control skin homografts (first set) were statistically indistinguishable on the 5th and 6th days. The scores for homografts placed after a single maximal antigenic stimulus ($20 \times 10^6$ homologous whole spleen cells, injected intraperitoneally) and excised on the 6th day were significantly smaller than the scores for those excised on the 5th day ($0.005 < P < 0.01$).

6. *Control values.* The mean score for control homografts on the 6th day for seven different experiments was 4.75 units (standard error, $\pm$ 0.174, $N = 55$). (This is equivalent to between 75- and 90-percent survival of epithelial cells.)

7. *Reproducibility of scoring method.* Seventy-five slides, chosen at random, were reexamined, and scores of the first and second readings were compared. A statistical analysis of the differences in paired data showed that there were no significant differences between the original and the repeat readings.

In Fig. 1, the injected doses are indicated by arrowheads above the abscissa; the units of the abscissa are the logarithms (base 10) of the number of injected cells. The mean scores for the individual test groups are shown by the crosses. The dose-response, represented by the solid line *AB*, was obtained by the method of least squares from the observed points (crosses); all but the highest dose ($6.3 = \log_{10} 2 \times 10^6$ cells), of which the mean score and variance were zero, were used. The dotted lines *mm'* and *nn'* are the confidence limits at $P = 0.05$ for the regression line (*not* for data for the individual groups). The other symbols (squares and circles) indicate the observed dose-response in two subsequent experiments. By means of statistical analysis, the regression is found to have a very high order of probability ($P < 0.001$) (*7*).

It is evident that a significant response can be measured over a 20-fold range in dose, linear with the logarithm of the dose in the range of 50,000 to 1 million cells (*8*).

LEWIS T. MANN, JR.
JOSEPH M. CORSON
GUSTAVE J. DAMMIN
*Departments of Pathology, Harvard Medical School* and *Peter Bent Brigham Hospital, Boston, Massachusetts*

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## Cytopathic Effect of Canine Distemper Virus in Tissue Culture

*Abstract.* A characteristic cytopathic effect was obtained with canine distemper virus (Onderstepoort strain) propagated in chick embryo fibroblast monolayer tissue culture. In limiting dilutions the lesions were focal. The titer at the 20th tissue culture passage was approximately $10^5$ $TCD_{50}$/ml. Cytopathogenicity was specifically inhibited by distemper immune serum. Minute plaques were produced under agar overlay.

Studies of canine distemper virus (CDV) have been handicapped by the lack of a tissue culture system in which the virus produces visible lesions. In addition to the veterinary importance of distemper virus, interest has been stimulated by the recently observed serologic relationship between CDV and measles virus (*1*).

Table 1. Titration of tissue-culture-adapted CDV in the chick embryo and in chick fibroblast tissue culture.

| Chick embryo CAM* ($ID_{50}$) | Tissue culture fluid medium (focal lesions) | Tissue culture agar overlay (pfu)† |
|---|---|---|
| *Tissue culture passage level:* 10 | | |
| $2.1 \times 10^4$‡ | $2 \times 10^5$ | $4.2 \times 10^5$ |
| *Tissue culture passage level:* 20 | | |
| $2.9 \times 10^4$ | $1 \times 10^5$ | $6.2 \times 10^5$ |

* CAM, chorioallantoic membrane. † pfu, plaque-forming units. ‡ Titer/1.0 ml.

Tissue cultures were prepared by trypsinization of 11-day-old chick embryos (*2*). The cells were suspended in Earle's saline containing 75 mg of $NaHCO_3$ per 100 ml, 0.25 percent lactalbumin hydrolyzate, and 4 percent calf serum. Ten milliliters of the cell suspension (900,000 cells/ml) were added to 4-oz prescription bottles, and confluent monolayers were obtained within 24 hours. The virus employed was the egg-adapted Onderstepoort strain (*3*) of CDV (208th embryo passage) (*4*). Tissue culture passage was initiated by inoculation of 1.5 ml of a stock CDV chorioallantoic membrane suspension (20 percent) which had been stored at −70°C. A control series of tissue cultures was initiated with an inoculum of uninfected chorioallantoic membrane. The cultures were inoculated at 24 hours and maintained in a medium consisting of 50 percent bovine amniotic fluid, 2 percent calf serum, and 48 percent Earle's saline, containing 75 mg of $NaCHO_3$ per 100 ml. This medium maintained cells in good condition for 3 weeks without further exchange. The early passages were harvested between the fifth and ninth days, and beginning with the 14th passage, on the third or fourth day. The cultures were harvested by scraping. The cells plus supernatant fluid were passaged without storage or freezing. Beginning with the 15th passage, the cytopathic effect was sufficiently rapid that the fibroblast monolayer could be maintained adequately in the original growth medium and therefore no exchange with maintenance medium was necessary. The present passage method (32nd passage) consists of the inoculation of 0.1 ml of undiluted supernatant plus cells simultaneously with the plating of the trypsinized chick cell suspension in the growth medium. The fibroblast monolayer forms within 24 hours and cytopathic effect appears in the formed sheet at 48 to 72 hours. Two of three other attempts to establish the Onderstepoort strain of CDV in this system were successful.