TABLE 3

DISTRIBUTION OF SKIN-SENSITIZING POWER IN HUMAN SERUM PROTEINS AFTER FRACTIONATION BY ELECTROPHORESIS CONVECTION

(As judged by sensitization tests with serial dilutions)

Fraction	Dilutability of original fraction	Dilutability of solution containing 1 mg/ml of		
		Total protein	Total globulin	β-globulin
Whole	256	3.7	7.4	13.2
Top 1	2	0.65	0.66	21.5*
Top 2	2	0.75	0.76	12.5
Top 3	< 1			
Top 4	< 1			
Top 5	< 1			
Top 6	24	5.7	6.3	11.4
Top 7	32	3.6	4.6	11.2
Bottom 7	4	0.09	0.47	3.0*
BA-7	1	0.02	0.19	*
BG-7	2	0.35	0.35	

* Electrophoretic area too small for reliable estimate.

roughly 0.1 mg β/ml, as determined from Tops 6 and 7, which transferred markedly in our subject when employed in their original concentrations. The feeble sensitizing qualities of all other fractions were, therefore, consistent with the concept of β-activity.

It will be remarked that Tops 3, 4, and 5 could not be used in the analysis of β-activity (Tables 2 and 3), since they transferred only questionably in E.M.M. From Table 1 it will be noted that the β-globulin concentration of Tops 3 and 5 approximated 0.1 mg/ml, the figure mentioned above as the borderline requirement. Top 4, with its content of 0.2 mg, should have produced detectable sensitization. Although it failed to do so in E.M.M., it transferred slightly in another recipient, resembling Tops 1 and 2 in potency, as might have been expected from its rather low β-content.

Since the writing of this paper, another serum containing reagins (for ragweed pollen) has been similarly fractionated and studied by the dilution technique. Activity appeared to be distributed through the γ- and β-globulins. The maximal volume in which 1 mg of total protein would still transfer sensitivity ranged from about 1 ml for Top 1, which consisted predominantly of γ-globulin, to approximately 4 ml for Top 7, the β-rich fraction. These results suggest that allergic activity is concentrated in β-globulin. However, it was not restricted to this protein as in the instance of our insulin-reaginic serum.

The pollen-reaginic serum also contained thermostable, or so-called blocking, antibody. Its presence in the fractions was judged by the amount of pollen antigen each could neutralize, heated samples being mixed with graded strengths of pollen extract for subsequent test in sensitized normal skin. Neutralizing activity was found in fractions rich in γ-globulin. Top 1 (consisting 91% of a slow γ-globulin having a mean mobility of -1×10^{-5}) possessed a neutralizing power of about 10 phosphotungstic-acid-precipitable N u pollen/mg total protein. Top 5 (containing 70% of fast γ-globulin, with a mean mobility of -2×10^{-5}) carried an inhibiting activity of over 30 u/mg in two test subjects. Antibody content appeared to be minimal in Tops 2 and 3, which were composed largely of γ-globulin of intermediate mobility. The remaining fractions, comprised almost exclusively of β-globulins, α-globulins, or albumin, showed negligible neutralizing power. We therefore conclude that the thermostable antibody is concentrated in the γ-globulins, with a bimodal distribution. The latter might be attributable to the existence of more than one antibody for the multiple antigens known to be present in pollen.

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Manuscript received July 7, 1952.

Effect of Halogens on the Production of Condensation Nuclei by a Heated Platinum Wire

Bernard Vonnegut[1]

General Electric Research Laboratory, Schenectady, New York

Experiments performed in this laboratory show that the presence of small traces of gaseous halogens or halogen-containing compounds in the atmosphere causes a very large increase in the rate at which a heated platinum wire produces condensation nuclei. The apparatus used in these experiments is shown in Fig. 1. Air from the room is drawn through a long filter of fine glass wool, which removes practically all condensation nuclei. This nuclei-free air is then passed through a chamber containing a platinum filament electrically heated to about 500° C and into an apparatus which measures the concentration of condensation nuclei. In this work an automatic condensation-nuclei-measuring device (*1*) was used, but less complicated equipment, such as an Aitken counter (*2*) or a simple expansion chamber, is satisfactory. When the platinum filament was first turned on, a large concentration of nuclei was produced. The formation of these nuclei apparently resulted from surface contamination of the filament, for after a few minutes of operation

[1] Present address: Physical Chemistry Group, Arthur D. Little, Inc., Cambridge, Mass.

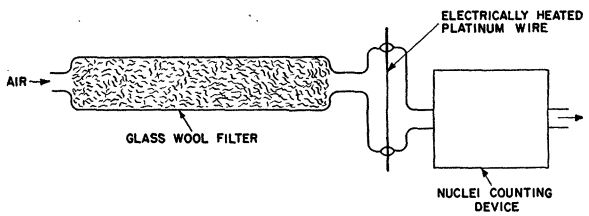

FIG. 1.

the filament ceased producing any nuclei, and the air entering the measuring device became nuclei-free.

It was observed that when small amounts of gaseous halogen or halogen-containing substances such as Cl_2, I_2, Br_2, and CCl_4 were present in the air being drawn through the filter the heated platinum filament produced large numbers of nuclei. The sensitivity of the apparatus to CCl_4 was found to approach that of the commercial halogen leak detector which operates on the positive ion emission of a heated platinum filament.

A possible explanation for this phenomenon is as follows. At 500° C the vapor pressure of platinum is so low that an insufficient concentration of atoms is introduced into the air to condense and form condensation nuclei. The halogen or halogen-containing gases in the air, which pass freely through the glass wool filter, react with the hot platinum surface. The resulting compounds, although relatively nonvolatile at room temperature, have a higher vapor pressure than the platinum and at 500° C are vaporized in sufficient concentration to condense upon mixing with air at room temperature to form large numbers of nuclei.

It is reasonable to suppose that other systems can be devised in which small concentrations of certain gaseous materials will result in the production of large numbers of nuclei from certain nonvolatile substances maintained at an elevated temperature.

The fact that nuclei having masses of the order of 10^{-17} g are readily detectable in concentrations as low as 10/ml suggests that very sensitive analytical techniques based on nuclei detection are feasible.

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Manuscript received July 10, 1952.

Analysis of Dose-Response in Relation to Mechanism of Pulmonary Tumor Induction in Mice

W. E. Heston and M. A. Schneiderman[1]

National Cancer Institute,
National Institutes of Health,
U. S. Public Health Service, Bethesda, Maryland

It is almost universally assumed that the transformation of cells to malignancy involves some change in the cell. The nature of this change remains one of the basic questions in cancer research. Berenblum and Shubik (*1*) have postulated a two-phase process, the initiative phase and the promoting phase, a concept that has been supported by others. Blum (*2*) has suggested that in the induction of skin tumors by ultraviolet irradiation there is progressive acceleration of growth by successive doses. This suggests that there may be successive changes in the cell.

Much consideration has been given to the somatic mutation theory of carcinogenesis proposed by Von Hansemann and later by Boveri, and recently vigorously supported by Strong (*3*) and others. Although the number of changes might not necessarily be limited to one, the present concept of this theory would tend to locate the change or changes in the nucleus, presumably as gene changes.

Interest in the somatic mutation hypothesis recently has been strengthened by the general search for a positive correlation between mutagenic and carcinogenic capacities of chemicals. An over-all positive correlation has not been observed, but isolated experiments testing related compounds under standardized conditions have presented positive correlations that in themselves suggest that possibly the change to malignancy is basically genic. Tests in this laboratory (*4, 5*) on the induction of pulmonary tumors in strain A mice with mustard compounds have shown that both the nitrogen mustard, methyl-bis (2-chloroethyl) amine hydrochloride, and sulfur mustard, bis (2-chloroethyl) sulfide, which Auerbach (*6*) and others have shown to be strong mutagens, were also potent carcinogens, whereas mustard oil, ethyl iso-thiocyanate, which was found to be a very weak mutagen, did not significantly increase the number of lung tumors.

In an analysis of the number of papillomas observed in mice painted repeatedly with Benzpyrene, Charles and Luce-Clausen (*7*) demonstrated a linear relationship when the square root of the number of papillomas was plotted against time, an expression of dose. This suggested the necessary occurrence of two separate events, or mutations, in the cell for the induction of a papilloma, the requirement if a recessive mutation were involved.

From our experience with pulmonary tumors in mice, it seemed desirable to analyze the pulmonary tumor response to graded doses of a carcinogen to ascertain whether here also would be found a parabolic curve indicating more than one change, or a straight line, as could be expected if only one change were necessary for a cell to give rise to a tumor. Certain outstanding advantages are offered by this type of tumor: (1) the many nodules appearing on the surface of the lungs afford a quantitative measure of response; and (2) a single dose of the carcinogen, even of very small amount, gives a measurable response. Thus, repeated doses such as were encountered in the studies of papillomas and Blum's radiation studies could be avoided.

[1] With the technical assistance of W. D. Levillain.