We are reporting here certain observations made upon dogs fed diets containing agenized (NCl_3-treated) proteins. These observations may help to identify the toxic compound or compounds and to determine human susceptibility.

Nitrogen trichloride (NCl_3) may be prepared by the interaction of chlorine gas and ammonium chloride solution. If the pH of the reaction mixture is raised above 4.5, increasing amounts of homologues of NCl_3 are formed, namely, $NHCl_2$ (dichloramine) and NH_2Cl (monochloramine) (*1*). It is of considerable theoretical interest that these latter chloramines *do not* produce in flour a detectable convulsant compound. Only trichloramine (NCl_3) is capable of reacting with protein to produce a convulsant agent which is active after oral administration.

A similar reaction product, identified by its physiologic and electroencephalographic effects, can be produced in the wheat proteins gliadin and glutenin, the corn protein zein, and the milk proteins casein and lactalbumin (*5, 8*). When similar amounts of these proteins have reacted with equal amounts of gaseous NCl_3, the resultant toxicity appears to be proportional to the sulfur content of the original protein and not to be correlated with any other portion of the amino acid composition of said proteins (*6*). If these agenized proteins are hydrolyzed by tryptic digestion, the soluble amino acid residues retain about 50% of the convulsive toxicity of the whole protein, and they appear to be more potent by the oral than by the intravenous route. A number of laboratories are now engaged in the attempted isolation of the altered amino acid or polypeptide responsible for the convulsions.

The effects of this unknown compound are as yet best analyzed by means of the electroencephalogram. An altered EEG appears before the overt convulsion, and the seizure pattern as seen with the EEG is characteristic and significantly different from that produced by other convulsants, e.g. Metrazol or strychnine (*7*). These characteristics form the basis for this communication. First, a seizure may be provoked in a susceptible animal (dog on a bleached diet for 3 days) by the inhalation of a mixture of 20% carbon dioxide and 80% oxygen. This is somewhat surprising in view of the known "depressant" effect of CO_2 on the cerebral and cerebellar cortex. Secondly, the seizure as recorded by the electroencephalogram, whether occurring spontaneously or provoked by CO_2 inhalation, is seen to arise in the cerebellum (or at least is recorded from cerebellar leads) a few seconds before the seizure is seen in the cerebral cortex. That the cerebellum has the capacity to convulse has been appreciated although little emphasized, but that it has the capacity to "drive" the cerebral cortex into a typical tonic-clonic seizure, or that both cerebrum and cerebellum are driven almost simultaneously from a subcortical locus, would tend to establish agene convulsions (along with DDT convulsions) as a unique entity in the much explored fields of human and experimental epilepsy.

If the reaction product between NCl_3 and the protein moiety can be identified, we shall be much closer to an understanding of how it can induce convulsions. Until that time we must speculate regarding the mechanism by which certain low-molecular-weight chlorinated compounds (e.g. DDT) can produce electrical changes in the cerebellum along with cerebellar degeneration (*2, 3*), and as to why the reaction of proteins with certain chloramines and not with others can produce seizures and degeneration in the cerebellum while making the cerebral cortex more susceptible to activation by CO_2. This may well be a case where intensive investigation of what is at first sight a purely nutritional problem will yield fruitful results in the analysis of fundamental neurophysiological mechanisms.

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Construction of Glass Diaphragm Leaks for Gas Analysis With a Mass Spectrometer

Vernon H. Dibeler[1] and T. Ivan Taylor[2]

Department of Chemistry, Columbia University

It has been shown (*1*) that the requirements of gas flow necessary to perform satisfactory gas analyses with a mass spectrometer are fulfilled essentially by molecular flow through a small hole in a thin diaphragm. For certain applications a properly designed capillary leak has also proved useful (*3*). One method of constructing a diaphragm leak has been described by Honig (*1*). We have devised a different technique which seems to be suitable for making a number of relatively uniform diaphragm leaks. Several of these have been prepared and installed in the gas inlet system of a Nier-type mass spectrometer.

The procedure used in making the diaphragm leaks is as follows: One end of a number of 5-cm lengths of 7-mm Pyrex tubing is turned in a medium oxygen flame until the tube is nearly closed and only a very fine capillary (0.02–0.04 mm in diameter) remains through the thickened end. While the end is still hot and workable, a loose-fitting carbon rod is inserted into the open end of the tube. The constricted end of the tube is gently

[1] National Institute of Health Research Fellow, 1947–48. Present address: National Bureau of Standards, Washington, D. C.

[2] The authors wish to thank R. B. Bernstein for help in performing the rate of effusion experiments.

pressed between the end of the rod on the inside and a carbon block held perpendicular to the axis of the tube on the outside (Fig. 1a). Excessive pressure at this point usually distorts the normally circular cross section of the capillary. Since the remaining procedure does not materially affect the diameter of the orifice, an estimate of the final orifice size can now be obtained with a microscope or jeweler's loupe and unsuitable closures set aside.

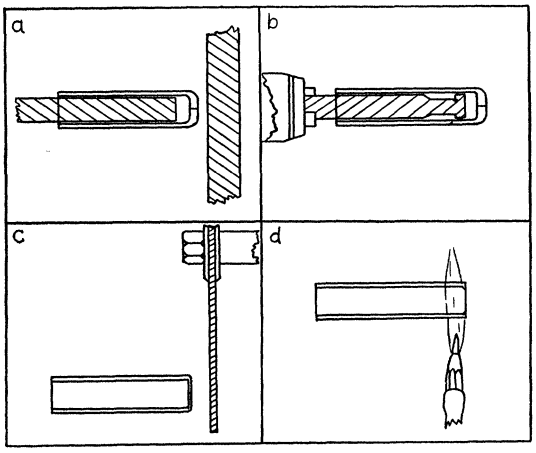

FIG. 1

Next, a length of drill rod approximately 5 mm in diameter is mounted in a lathe or drill press and the end flattened with a lathe tool or grinder. Using approximately 200-mesh carborundum dust, the inner surface of the constricted end of the tube is ground against the end of the drill rod until any conical portion of the capillary is removed (Fig. 1b). The inner face is now semipolished in the same manner, using fine alumina (Gamal No. 2) or rouge. If the shank of the grinding tool is only slightly smaller than the inside diameter of the tubing, the inner face of the diaphragm will be very nearly square with the wall, even though the tubing is held by hand while grinding. The outer face of the diaphragm is now ground down to a thickness of less than 0.1 mm. A metallographic polisher of a radial glass-cutting saw with a 200-mesh carborundum disk is quite useful for this purpose (Fig. 1c). The tubing is held by hand on the movable work guide with a slight pressure against the side of the disk. Care must be taken to maintain the axis of the tube perpendicular to the disk surface at all times, and frequent inspection with a lens is necessary to prevent undergrinding or destroying the diaphragm. Diaphragms set aside because of unsatisfactory closure in the first step are useful for practice grinding, and short experience makes the above procedure relatively easy. After rough grinding, the diaphragm is carefully hand-lapped, using Gamal No. 2 or rouge. It was our experience that a diaphragm thickness of about twice the diameter of the hole would not remain plugged with polishing compound when subjected to a small hydrostatic pressure. This was used as a rough indicator for the proper amount of grinding. The semipolished diaphragm is now boiled in concentrated HCl and rinsed in distilled water and alcohol. When dry, its thickness is estimated by focusing a microscope first on its upper and then on its lower surface, using a small scratch on each surface for reference. The vertical movement of the barrel is read on the vernier scale of the microscope. If no further grinding is necessary, the diaphragm is fire-polished by directing a soft oxygen flame on the side wall of the tubing near the diaphragm end (Fig. 1d). Heat sufficient only to polish the diaphragm and adjacent side walls does not distort or enlarge the hole within the limits of measurement and results in a polished, flat diaphragm capable of withstanding a pressure differential of at least 1 atm. The diameter of the finished leak is measured microscopically.

Dimensions of one lot of 5 leaks are given in columns 2 and 3 of Table 1. Leak No. 5 was broken, and the diaphragm thickness was carefully measured microscopically and found to be quite uniform. The other diaphragms were estimated, as described above, to be about the same thickness.

TABLE 1

NITROGEN AT .05 mm, 300° K

Leak	Diameter (mm)	Thickness (mm)	Effective area, A* (cm^2)	Effusion (mg/day)
1	.044	†	5.45×10^{-6}	.42
2	.044	†	5.45 "	.42
3	.040	†	4.22 "	.33
4	.032	†	2.40 "	.19
5	.040	.088	4.22 "	.33

† Estimated to be 0.09 mm by microscopic examination.

The flow of gas through an orifice in a diaphragm of finite thickness may be calculated from the following relations, provided the pressure is sufficiently low so that the mean free path of the molecules is larger than the diameter of the hole. The total number of molecules, N, effusing from the orifice is given by the number of molecules, Z, which strike a unit area of surface per second, multiplied by the area, A, times a correction factor, P. Thus, $N = PAZ = A^* Z$. The value of Z is given by $Z = 3.537 \times 10^{22} p/(MT)^{\frac{1}{2}}$ molecules/sec, cm^2, where p is the pressure in millimeters of mercury, M is the molecular weight of the gas, and T, the absolute temperature. Loeb (*2*) has tabulated values of P as a function of the ratio of the thickness of the diaphragm, L, and the radius of the hole, R. The calculated effective area, A*, of each orifice is given in column 4, Table 1. With the first four leaks in parallel, the total effective area is calculated to be 1.75×10^{-5} cm^2. The uncertainty is estimated to be ±20%, principally because of uncertainty in the thickness of the diaphragm. The mass of nitrogen that would effuse through the four orifices from a reservoir at a pressure of 0.05 mm and a temperature of 27° C is 1.36 mg/day. For a 10-liter reservoir this is equivalent to a pressure drop of approximately 6%/hr.

The four leaks in parallel were sealed into the inlet system of a mass spectrometer. Several tests were made in which the rate of pressure change in a 10-liter reservoir behind the leaks was periodically measured, both by means of a McLeod gage and by detection of the appropriate ion with the mass spectrometer. The results indicated an effective area of 2.1×10^{-5} cm for the four leaks in parallel and a nitrogen flow of about 1.6 mg/day, agreeing with the calculated values within the estimated uncertainty.

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A New Influence on Chemically Induced Sarcomata[1]

LEONELL C. STRONG

Yale University School of Medicine

Many influences have been established as effecting the induction of sarcomata by chemical means. These influences may be classified into two groups, as follows: (1) the environmental and (2) the genetic. Under the environmental influences may be listed the specific carcinogenic compound, the amount used or dosage, the vehicle or solvent, the mode of introduction, the age of the experimental animal, and the species or subline of experimental animal employed. The genetic influence has always been inherited as a dominant multiple factor complex, although, in many cases, the susceptibility of the F_1 individual to the chemically induced tumor approaches the susceptibility of the dominant parent, but does not do so completely.

Examples of linkage between genes for susceptibility to induced neoplasms and the well-known color or morphological genes of mice have been demonstrated primarily by the work of Heston and of Strong. In an attempt to investigate further the nature of the genetic influence on the induction of tumors by methylcholanthrene, the following experiment was performed:

Mice of the NHO strain showing an intermediate degree of susceptibility to the subcutaneous development of sarcoma at the site of the injection of methylcholanthrene were outcrossed to mice of the C57 subline which showed a high degree of susceptibility to the same induced tumor. The outcross was made in both directions. Two hundred fifty-four F_1's were obtained. At 60 days of age the F_1 mice were injected with 1 mg of methylcholanthrene dissolved in 0.1 cc of sesame oil. The mice were periodically examined for tumors. Data obtained on the rate of appearance of tumors induced by methylcholanthrene are plotted in Fig. 1.

The mice were divided into three groups according to the litter in which they were born. Mice born in the first and second litters comprise group 1; mice born in the third and fourth litters of the same breeding parents were put into group 2; and mice born in the fifth and sixth litters of the same parents were classified as group 3. The three groups were approximately of the same

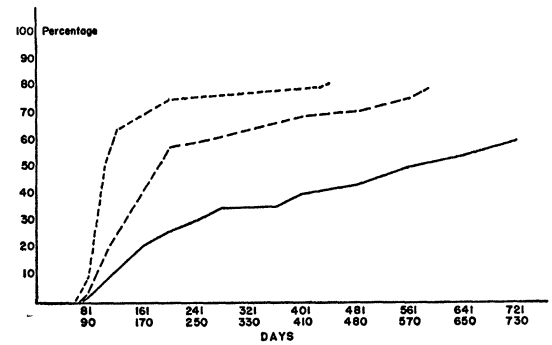

FIG. 1. Data obtained on the rate at which fibrosarcomata appear at the site of injection of methylcholanthrene in a series of F_1 mice. Time in days is given on the base line, the percentage of mice showing tumors along the vertical line. Group 1 consists of mice belonging to first and second litters (solid line); group 2, of mice of the third and fourth litters (long-dash line); and group 3, of mice from the fifth and sixth litters of the same breeding parents (short-dash line).

size (93, 80, and 81 mice, respectively). An examination of Fig. 1 discloses the fact that mice of the three groups develop sarcomata at the site of the injection of methylcholanthrene at significantly different rates. The rate for the appearance of tumors was slowest in group 1, intermediate in group 2, and highest in group 3.

All mice of the F_1 generation produced by an outcross of mice of two inbred strains are theoretically genetically alike. The difference of susceptibility to induced tumors obtained in this experiment is consequently not a genetic one. It is obvious that something which is increasing or decreasing in the mother's body is being handed down to her offspring. It is also possible that this principle varies in the father's contribution to progeny, although the evidence for this concept has so far not been indicated. This transmitted principle sensitizes or changes the progeny's susceptibility to a subsequent injection of methylcholanthrene, influencing the rate at which the offspring develop sarcoma in the presence of a given amount of methylcholanthrene. There are possible at least three modes of transmission for this principle. It may be (1) by cytoplasmic inheritance, (2) by transplacental transmission, or (3) through the mother's milk. These three were the modes of transmission regarded as possible for the transmission of susceptibility for spontaneous adenocarcinoma of the mammary gland in mice by the group at the Jackson Memorial Laboratory. In the spontaneous adenocarcinoma work it was subsequently conclusively demonstrated that the principle was transmitted through the mother's milk. This principle producing adenocarcinoma of the mammary gland is now sometimes referred to as the virus of Bittner.

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