despite the extensive national publicity. At the 130 centers reporting inquiries, 49 did no chromosome studies (Table 2). A median of 4.6 working days was spent on this issue by the centers reporting inquiries (Table 3).

No direct beneficial effects of this episode for those who were counseled were reported, although one person suggested that an indirect benefit had been to make individuals more aware of genetic counseling services. With regard to other outcomes, a total of 11 amniocenteses were reported from eight centers. In one woman, increased chromosome breakage was reported in cells cultured from amniotic fluid (an observation which may have been due to viral contamination) and an elective abortion was done (*6*). Three centers reported that they were aware of a total of eight women who, without first undergoing diagnostic amniocentesis, elected to have abortions because of concern about their exposure to spray adhesives.

These data are minimum estimates of the impact of this issue. They do not include results on women who may have consulted family physicians or obstetricians but were not referred to genetic centers. They do not include data from genetic units not listed by the directory (although we are unaware of any, and it seems that the directory lists many inactive individuals), nor do they include the experience of the five centers that did not reply (*7*). Moreover, there is no estimate of the nature, extent, or consequences of anxieties created by this issue, but we are aware of no ready measure of these.

The centers reporting the greatest number of queries were in Minnesota (where many of the substances were made, and where there apparently was heavy industrial exposure) and, for unexplained reasons, the Pacific Northwest. One respondent noted that a local newspaper had given the issue extensive publicity, which may have prompted many inquiries to his unit. A further study we carried out in New York State suggested that centers receiving no inquiries concerning spray adhesives see fewer patients for genetic counseling of any type than those who reported receiving queries (*8*). In addition we analyzed the results in a subset of the total of 182 active independent centers: 36 major genetic units known through their publications to be active in genetic counseling. The median (7.5) and mean (16.9) number of inquiries at these were more than double the experience (3.2 and 6.8, respectively) in the total group of 182 active independent centers replying. Only 1 of the 36 major centers reported receiving no inquiries, compared to 52 of the total 182. Thus, at least some of the factors affecting the number of queries received appear likely to be the location of the target population, variation in local publicity, and the size of the referral population of the centers.

Table 3. Frequency of working days expended at 130 active centers receiving inquiries concerning spray adhesives.

Frequency of working days	Centers (No.)
0	12
1 to 5	54
6 to 10	31
11 to 15	9
16 to 20	2
21 to 25	0
> 25	14
Some	5*
No estimate provided	3*
Total	130
Range	0 to 160
Mean †‡	10.43
Quartiles †	
25 percent	2.4
50 percent (median)	4.6
75 percent	9.4
Total working days (minimum)§	1273

*Of the 130 independent active centers receiving inquiries, there were eight nonrespondents concerning working days of which five reported doing chromosome studies and three did not report any such studies. We have classified the five which reported doing cytogenetic studies as expending "some" working days on this issue. †The calculation of mean, quartiles, and minimum total working days is for the 122 centers providing exact estimates. ‡Standard deviations were not calculated because of the skewed distribution observed. §At centers providing exact estimates.

Variation in the belief of the evidence cited as supporting toxicity and the extent of the anxiety expressed by the counselees may have contributed to the variation in the total number of chromosome studies, but we have no direct data on this.

The possibility that any substance to which there is extensive population exposure may be teratogenic or mutagenic is, of course, a real one, and the report of suspicion of an effect should be taken seriously. The consequences of the episode reported here, however, illustrate the need to distinguish suspicion of toxicity from evidence for toxicity. If there is nationwide publicity concerning possible mutagenic or teratogenic hazards of a substance, the recent legalization of abortion in the United States and the ready availability of prenatal diagnostic procedures make it likely that many women will avail themselves of amniocentesis (if it appears appropriate to the suspected outcome) and, even in the absence of definitive information, will abort fetuses they believe to be at risk.

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5. Our questionnaire requested that, if two or more affiliated individuals received an inquiry from us, one of them would prepare a summary of their center's experience. In one instance, however, separate replies were received from two individuals apparently affiliated with the same unit, without indication as to whether their reports overlapped. In this case, only the reply of the individual reporting the greater number of queries was included in the tabulations, because of the possibility that the response of the other was a subset of the one tabulated.
6. The genetic counselor informed the mother that, on the basis of this evidence, he was unable to make any specific prediction about the state of the fetus. The mother elected to undergo an abortion, but she did not inform the counselor of this decision until after the procedure, when the abortus had been fixed in formalin. Thus further chromosome studies could not be done. Detailed pathologic examination of the abortus at another institution revealed no evidence for any congenital defect (J. Q. Miller, personal communication).
7. No reply was received from the center at which the association was first reported. It appears likely that extensive work was performed here because of the suspected toxicity of spray adhesives (*1–3*).
8. Out of a total of 27 active independent genetic counseling centers in New York State who replied, 18 reported receiving inquiries concerning spray adhesives and 9 reported receiving none. In a further investigation 16 of these 18 and 8 of the 9 were able to estimate the number of clients per year they counseled. The mean ± standard deviation (and median) were 204.8 ± 166.1 (175) and 136.3 ± 103.2 (150), respectively, somewhat higher in the group receiving inquiries concerning the adhesives, albeit not significant at the 5 percent level ($P \sim .3$).
9. We thank those who responded to the questionnaires and M. Hoff for advice on statistical matters.

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Carotid Body in the Sudden Infant Death Syndrome

Abstract. *Sixty-three percent of victims of the sudden infant death syndrome had a subnormal volume and 23 percent an enlarged volume of glomic cells in their carotid bodies. Evidences of antecedent chronic alveolar hypoxia and hypoxemia were found in both groups but were more severe in the victims with enlarged glomic tissue.*

Prolonged apneic periods during sleep have been described in several sudden infant death syndrome (SIDS) victims prior to death (*1, 2*). Such apneic episodes may be a common final pathway of death in SIDS. In several adult disorders such episodes of sleep apnea are associated with chronic alveolar hypoventilation (*3*). Many SIDS victims show characteristic consequences of such chronic hypoventilation,

Table 1. The volume and volume to body weight ratio of glomus cells in the carotid bodies of infants from 1 to 10 months of age. The numbers of cases are shown in parentheses. All mean values ± S.E.

Age	Volume carotid glomus cells in controls (mm^3)	Ratio: $\frac{\text{Volume carotid glomus cells } (mm^3)}{\text{Body weight (kg)} \times 10^{-1}}$		
		Controls	SIDS without infection	SIDS with infection
1 to 2.4 months	0.46 ± .06	0.84 ± .07 (8)	0.43 ± .03*(17)	0.73 ± .12 (6)
2.5 to 5 months	0.58 ± .10	0.86 ± .08 (7)	0.21 ± .04†(10)	0.65 ± .10(13)
5.1 to 10 months	0.69 ± .11‡	0.82 ± .06(11)	0.10 ± .03† (4)	0.32 ± .13*(6)

*$P < .025$ compared with controls of same age. †$P < .005$ compared with controls of same age. ‡$P < .05$ compared with controls 1 to 2.4 months old.

including increased muscle in the pulmonary arteries and an abnormally heavy cardiac right ventricle (*4, 5*). Hypoventilation leads to hypoxemia and many SIDS victims show such evidence of chronic hypoxemia as an increased mass of adrenal chromaffin tissue, an abnormal proliferation of brain stem astroglial fibers, and an abnormal retention of extramedullary erythropoiesis and brown fat (*6, 7*).

We searched for carotid body abnormalities in SIDS victims. A defect in the chemoreceptor function of the organ might contribute to abnormalities in ventilatory control in SIDS. The organ might also be enlarged in SIDS victims, since it characteristically responds to chronic hypoxemia by developing a hyperplasia of its glomic cells (*8*).

Well-nourished infants who died between 1 and 10 months of age and had well preserved tissues were used in the study. Seventeen males and 14 females were classified as noninfected SIDS victims when death was sudden, completely unexpected, and unexplained by any routine clinical or postmortem findings. Their mean age at death was 2.4 ± 0.2 (S.E.) months. Another 14 males and 11 females were classified as SIDS with infection since they had tracheobronchitis, pneumonia, laryngitis, or otitis media of too mild a degree to explain death (*4, 5*). Their mean age at death was 3.4 ± 0.2 months. Fourteen males and 12 females were classified as controls. They had no evidences of chronic hypoxia, hypoxemia, or infection, and most were victims of accidents, fires, or homicides. None lived longer than 4 hours after the acute event that led to death. Their mean age at death was 3.8 ± 0.4 months.

The carotid bifurcations were dissected out at autopsy and fixed in buffered 10 percent formalin. Serial sections 5 μm thick were prepared, and sections at intervals of 50 μm were stained with hematoxylin and eosin. The carotid body is a mixture of glomic cells, neural tissue, blood vessels, and other elements. The total volume of the glomic cells, their number, and individual cytoplasmic volume were determined for each organ by point counting and the use of Simpson's rule as quoted by Dunnill (*9, 10*). Nerve fibers in the carotid body were stained by the method of Sevier and Munger, and the volume was quantitated in the same manner as the glomic tissue (*11*).

The free walls of the two ventricles of each heart were dissected from the interventricular septum and separately weighed. These weights were calculated as a proportion of body weight (*5*). The resultant values did not change in the controls between 1 and 6 months of age, so direct comparisons were possible between groups within this age range (*5*). The point counting method was used to quantitate the amount of muscle in pulmonary arteries whose diameter was 100 μm or less (*4*). The ratio of area of arterial media to area of arterial intimal nuclei was used as the measure of arterial muscle mass (*4*). A minimum of 20 arteries was measured in each infant and a mean figure was recorded as the value for the case. These values did not change between 1 and 10 months of age in the controls. Extramedullary erythropoiesis was recorded when nests of erythropoietic cells were identified between the cords of hepatic parenchymal cells. Hepatic erythropoiesis is considered an abnormality in any infant after the newborn period (*6*).

Periadrenal brown fat is normally replaced by white fat during the first year of postnatal life. It has recently been found that brown fat is replaced more slowly than is normal in infants who are chronically hypoxemic after birth and in SIDS victims (*6*). The proportion of fat cells that were brown was determined in each of our subjects by the point counting method (*6, 10*). The transformation of brown fat cells to white cells is not normally advanced until after 4 months of age, so only infants 5 months of age and older were included in the fat analyses (*6*). All measurements in the study were made by an examiner without knowledge of an infant's age or diagnosis. Student's *t*-test, chi-square, and linear regression analyses were used in the study.

The volume of carotid body glomic tissue increased with age in the controls (Table 1). The volumes of glomic tissue were calculated as a proportion of the infants' body weights. The resultant values did not change significantly between 1 and 10 months of age in the controls, so direct comparisons were possible between various diagnostic categories without reference to age (Tables 1 and 2). Glomic volume to body weight values were significantly smaller in the noninfected SIDS victims than in the controls (Table 1). Smaller differences were found between infected SIDS victims and the controls (Table 1). The controls had 2.6 times as many carotid body glomic cells as did age-matched noninfected SIDS victims. Glomic tissue in the controls had a mean of 12 percent more cytoplasm per cell than did such tissue in age-matched noninfected victims.

One group of SIDS victims had a subnormal and another an enlarged volume of carotid body glomic tissue. Glomic tissue volume was classified as enlarged when the glomic volume to body weight ratio was in the upper 10th percentile of values for the control infants, that is, greater than 1.03 (Table 1). The 13 SIDS victims in this cat-

Table 2. The relation of the carotid glomus cell volume to body weight ratio (CG/BW) to various markers of chronic hypoventilation and hypoxemia.

CG/BW	Muscle of pulmonary arteries*	Relative weight right ventricle	Brown fat‡	Hepatic erythropoiesis§	Number of cases
		SIDS without infection			
< 1.03‖	4.7 ± 0.3¶	0.38 ± .03¶	30 ± 7%**	1/25 (4%)††	25
> 1.03	7.3 ± 0.5	1.20 ± .08	64 ± 6%	3/6 (50%)	6
		SIDS with infection			
< 1.03	5.0 ± 0.3**	0.93 ± .04	27 ± 3%††	1/18 (6%)	18
> 1.03	6.1 ± 0.3	1.10 ± .03	37 ± 4%	1/7 (14%)	7
		Controls			
< 1.03	4.1 ± 0.4	0.76 ± .03	13 ± 2%	0.22 (0%)	22
> 1.03	4.3 ± 0.3	0.79 ± .05	14 ± 2%	0.14 (0%)	4

*Ratio of the area of the arterial media to the total area of the intimal nuclei (mean ± S.E.). †Ratio of the weight of the right ventricle to the body weight (mean ± S.E.). ‡Percent (± S.E.) of periadrenal fat cells that are brown in type. §Proportion of cases that have hepatic erythropoiesis. ‖Ratio of the volume of carotid glomus cells in cubic millimeters to one-tenth of the body weight in kilograms. ¶$P < .005$ by comparison with paired value. **$P < .05$. ††$P < .01$.

egory had a larger mass of muscle in their pulmonary arteries, heavier cardiac right ventricles, and more retained brown fat and hepatic erythropoiesis than did SIDS victims with a smaller volume of glomic tissue (Table 2). The glomic tissue volume of 63 percent of the SIDS victims was classified as subnormal because their ratios of glomic volume to body weight were in the lower 10th percentile of values for the control infants, that is, less than 0.64. SIDS victims in this category had no significant correlations between the ratios of glomic volume to body weight and the various parameters of chronic hypoventilation and hypoxemia. Mean values for the ratio of volume nerve fibers to volume carotid body glomic tissue were similar in age-matched SIDS victims and controls.

Our study indicated that 63 percent of victims of SIDS has a subnormal volume and 23 percent an enlarged volume of glomic tissue in their carotid bodies. These abnormalities were not due to a failure of nerve fibers to grow into the organ. Evidences of antecedent chronic alveolar hypoxia and hypoxemia were found in both groups but were more severe in the victims with the enlarged glomic tissue. Neither of the carotid body abnormalities in the SIDS victims are easy to interpret because it is not known which structures in the organ comprise the various functional components of the chemoreceptor apparatus (*12, 13*). Glomic tissue is often hyperplastic in animals and human beings who are chronically hypoxemic, but it is not known whether this change is a primary or a secondary response to the blood gas abnormality (*8, 12*). The defect that is responsible for the subnormal volume of glomus cells in many SIDS victims might be primary in the organ or it could reside in brain stem respiratory centers and their efferent or afferent connections to the carotid body. Findings in our study give little basis for choice. However, the finding of both hyperplastic and hypoplastic glomic tissue in various SIDS victims increases the possibility that diverse mechanisms are involved in their deaths, even if apneic episodes remain a common final pathway.

A subnormal volume of carotid glomic tissue was less common in infected than in noninfected victims. In addition, there are other differences between the two groups. Infected victims are older at death, have smaller thymus glands, and have somewhat milder features of chronic hypoventilation and hypoxemia than do noninfected victims (*5*). This might suggest that a smaller proportion of deaths in infected victims are due to the hypoventilation-apnea mechanism. However, infections increase both the frequency and the duration of apneic episodes during sleep in susceptible infants, so abnormalities in respiratory control remain a likely explanation for many deaths in both infected and noninfected victims (*2, 14*).

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Proviral DNA of Moloney Leukemia Virus: Purification and Visualization

Abstract. *Closed-circular proviral DNA of Moloney leukemia virus has been purified from a 10^7 excess of cellular and mitochondrial DNA. The DNA can be visualized in the electron microscope and has the contour length of a molecule with a molecular weight of about 5.5×10^6. Electron microscopic observation of a hybrid between viral RNA and this circular DNA confirms the viral origin of this molecule.*

The viral DNA of RNA tumor viruses is found in several forms shortly after infection of a susceptible cell. Of great interest is a closed-circular, double-stranded superhelical form of the viral DNA shown to exist in cells infected by Rous sarcoma virus (*1*) or Moloney leukemia virus [M-MuLV (*2*)]. Its characterization has been impeded by the low amounts of this DNA that can be isolated from infected cells.

Nine hours after infection by M-MuLV, one closed-circular molecule of virus can be isolated per ten cells in a culture in which all the cells have been productively infected. This proviral DNA is therefore present with a 10^7-fold excess of nuclear and mitochondrial DNA (mtDNA). Several procedures have been used for the enrichment and purification of the viral DNA (*1, 2*). The Hirt extraction procedure (*3*) followed by centrifugation in a mixture of ethidium bromide and cesium chloride (EtBr-CsCl) (*4*) afforded a 700-fold enrichment. These steps leave the closed-circular viral DNA still contaminated by more than a 10,000-fold excess of mtDNA, whose similar closed-circular structure results in its copurification in the isopycnic centrifugation.

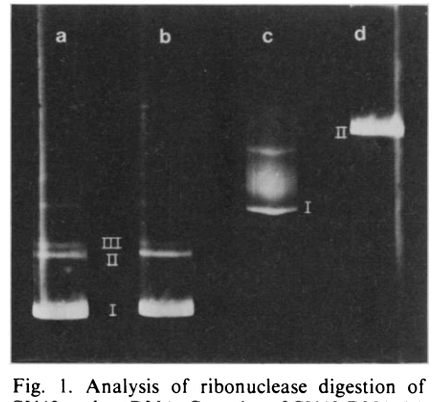

Fig. 1. Analysis of ribonuclease digestion of SV40 and mtDNA. Samples of SV40 DNA (a) and mtDNA (c) were subjected to electrophoresis directly in agarose gels (*15*). Equivalent samples were digested with ribonuclease A (50 μg/ml, 1 m*M* tris, 0.5 m*M* EDTA, *p*H 7.4, 1 hour at 37°C) before electrophoresis on parallel gels (b and d). (I) closed-circular DNA; (II) nicked-circular DNA; (III) linear DNA.

We have developed two methods for the further enrichment of the viral DNA: one generally applicable to all such closed-circular DNA's and the other particularly useful for the M-MuLV genome studied here. The first procedure depends on the existence of ribonucleotide linkages that exist in mtDNA (*5*). Analysis in an alkaline sucrose gradient has shown that these are absent from most if not all closed-circular proviral DNA molecules (*1, 2*). We also rely on the sensitivity of duplexed RNA to ribonuclease when digestion is performed at low ionic strength (*6*). Digestion of mtDNA with ribonuclease in low ionic strength should nick the closed-circular form of this DNA at any ribonucleotide linkage, and leave intact other closed-circular molecules lacking this linkage.