bodies has been shown to be effective in the blockage of cytotoxicity. This blockage apparently occurs during the last step of the immunological response as a result of the fixation of cytotoxic antibodies and paralysis of primed effector cells. Currie and Basham (*5*) demonstrated this blocking phenomenon in vivo by showing an increase in cell mediated cytotoxicity after tumor resection, and by washing lymphocytes in vitro to remove the attached tumor antigen or antigen-antibody complexes, thus restoring their cytotoxicity. Gentile and Flickinger (*6*) have demonstrated the presence of circulating breast tumor antigens and antibody complexes of this type in the serums of breast cancer patients. Calafat *et al.* (*7*) have shown that the redistribution and shedding of tumor antigens can be induced by the fixation of antibodies, and that this phenomenon results in an antigen-denuded cell which is not recognized by a second wave of effector agents.

Our studies confirm the antibody-induced redistribution and shedding of cell surface antigens, and show that this mechanism is active in our breast tumor system. The finding that after complete shedding the antigen was not replaced within 26 hours could indicate a basic method of tumor survival, since the antigen-denuded cell was viable and able to replicate but was not recognized by subsequent effector agents. Preliminary studies in our laboratory indicate that the speed and efficiency of antibody-induced antigen shedding may be related to immunoglobulin type. It appears that antibodies of the type that do not bind complement are redistributed at a different rate than complement-binding antibodies and are more slowly shed.

These findings suggest that tumor success or failure in the host is a complex and dynamic series of events, and that attention must be directed to tumor cell defense mechanisms in concert with the study of host defense mechanisms.

ROBERT E. NORDQUIST
Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, and
Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City 73190
J. HILL ANGLIN
Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center
MICHAEL P. LERNER
Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center

22 JULY 1977

References and Notes

1. G. C. Davey, G. A. Currie, P. Alexander, *Br. J. Cancer* **33**, 9 (1976); E. J. Leonard *et al.*, *Immunological Aspects of Neoplasia* (Williams & Wilkins, Baltimore, 1975).
2. R. E. Nordquist, D. R. Ishmael, C. A. Lovig, D. M. Hyder, A. F. Hoge, *Cancer Res.* **35**, 3100 (1975).
3. S. P. L. Leong, C. M. Sutherland, E. T. Krementz, *ibid.* **37**, 293 (1977).
4. E. Yefenof, I. P. Witz, E. Klein, *Int. J. Cancer* **17**, 633 (1976).
5. G. A. Currie and C. Basham, *Br. J. Cancer* **26**, 427 (1972).
6. J. M. Gentile and J. T. Flickinger, *Surg. Gynecol. Obstet.* **135**, 69 (1972).
7. J. Calafat, J. Hilgers, W. J. Van Bitterswijk, M. Verbeet, P. C. Hagman, *J. Natl. Cancer Inst.* **56**, 1019 (1976).
8. We thank H. K. Geis, P. L. Munson, and P. J. Riggs for their excellent assistance. Supported by American Cancer Society grant BC-230 and the Maizie Wilkinson fund.

11 January 1977

Impaired Regulation of Alveolar Ventilation and the Sudden Infant Death Syndrome

Abstract. *Infants who subsequently died of sudden infant death syndrome manifest alveolar hypoventilation during quiet sleep and abnormality of ventilatory response to carbon dioxide breathing in comparison to normal infants.*

Pathological evidence in infants dying of sudden infant death syndrome (SIDS) suggests that chronic hypoxia had been experienced (*1*). Physiological data have indicated prolonged sleep apnea (PSA) (*2*). Aaron *et al.* have suggested that the underlying mechanism might be abnormal cardiac conduction—prolongation of the repolarizing phase of cardiac electrical activity (QT interval) (*3*). In the course of conducting studies on infants who experienced aborted SIDS, we have obtained data on three who subsequently died of SIDS. We now compare the results of studies of these infants to those of normal controls. These studies were approved by the Human Studies Committee of the Massachusetts General Hospital. Written informed consent was obtained from parents.

The patients were three infants (two males) who were first seen at ages 7, 8, and 36 weeks after having experienced two, one, and multiple resuscitations, respectively. Chest radiographs, electrocardiograms, electroencephalograms, serum electrolytes, sugar, calcium, phosphorus, magnesium, and amino acids were normal.

The corrected QT interval (QT_c) was determined from the average of six randomly selected electrical complexes in standard lead II of electrocardiograms of awake infants and compared to 46 normal control infants whose ages spanned those of the study infants. The interpreter was unaware of the origin of each recording.

Ventilation at rest during quiet sleep and the ventilatory response to breathing 5 percent CO_2 in air were measured in each infant by the nasal pneumotachograph technique (*4*) at least 1 week after resuscitation. The results are compared with those for 12 normal infants of similar age also studied during quiet sleep.

Death occurred at 12, 36, and 39 weeks, respectively, when resuscitative efforts with bag and mask, initiated by the parents at home in response to an alarm from an electronic monitor, failed to restore vital functions. Patient 1 had experienced five and patients 2 and 3 had each undergone more than ten resuscitations prior to the final unsuccessful efforts. Autopsies of two failed to reveal a cause of death; an autopsy was not permitted on the third.

The QT_c interval was 0.39 seconds in

Table 1. The corrected QT interval in 3 SIDS infants compared with that in 46 normal infants. Mean ± standard deviation is presented for the normal infants.

Subjects	QT_c
Patient 1	0.38
Patient 2	0.40
Patient 3	0.40
Normal infants	0.39 ± .02

Table 2. Ventilation ($\dot{V}_E$) and the response to CO_2 breathing in 3 SIDS infants and 12 normal infants.

Subjects	$\dot{V}_E$ (ml min^{-1} kg^{-1}) (BTPS)	End tidal P_{CO_2} (mm-Hg)	$\Delta\dot{V}_E/\Delta P_{CO_2}$ (ml min^{-1} kg^{-1} mm-Hg^{-1})
Patient 1	215	40	20
Patient 2	177	37	20
Patient 3	112	44	10
Controls	206.4 ± 68.8	35.1 ± 1.9	63.1 ± 19.1

the three patients and 0.39 seconds in normal infants (Table 1). Thus, there was no evidence that an abnormal QT_c interval contributed to death of these patients.

End-tidal P_{CO_2}, reflecting the extent of alveolar ventilation, was 40, 37, and 44 mm-Hg, respectively, compared with 35.1 ± 1.9 in normal infants (Table 2). The change in minute ventilation [BTPS (*5*)] was corrected for body weight to permit comparison. The response to CO_2 breathing in each affected infant was less than the mean minus 2 standard deviations for normal infants.

Therefore, these three infants manifest alveolar hypoventilation and impaired regulation of ventilation. This finding is similar to the physiological defect in two infants with congenital alveolar hypoventilation who exhibit no response to CO_2 breathing, maintain normal ventilation while awake, but require mechanical ventilation to support life during sleep (*6*). We have not defined the cause of abnormal ventilatory control in any of these five patients. The data indicate that impaired regulation of alveolar ventilation rather than impaired cardiac conduction was the cause of death in the three infants of the present study. Alveolar hypoxia resulting from hypoventilation could explain the pathological findings described by Naeye in SIDS (*1*).

D. C. SHANNON
D. KELLY

Massachusetts General Hospital, Boston 02114, and *Department of Pediatrics, Harvard Medical School, Boston 02115*

References

1. R. L. Naeye, *N. Engl. J. Med.* **289**, 1167 (1973); *Science* **136**, 837 (1974); _____ and J. Dragge, *Pediatr. Res.* **9**, 298 (1975).
2. A. Steinchneider, *Pediatrics* **50**, 646 (1972).
3. B. J. Aaron, R. E. Goldstein, C. E. Clarck, R. S. Fisher, S. E. Epstein, *Circulation* **54**, 423 (1976).
4. S. A. Fagenholz, K. O'Connell, D. C. Shannon, *Pediatrics* **58**, 31 (1976).
5. Body temperature, (ambient) pressure, saturated (with water).
6. D. C. Shannon, D. W. Marsland, J. B. Gould, B. Callahan, I. D. Todres, J. Dennis, *Pediatrics* **57**, 342 (1976).

26 April 1977

Goblet Cells in Embryonic Intestine: Accelerated Differentiation in Culture

Abstract. *If duodenal tissue from 14-day chick embryos is cultured in chemically defined medium, more than twice as many goblet cells appear within 48 hours as in intact embryos during the same time. The increase in goblet cell number is further accelerated by 10^{-9} molar thyroxine but is prevented by 10^{-6} molar hydrocortisone. The results suggest that differentiation of intestinal epithelium is regulated in part by a circulating inhibitor.*

During the third week of development in ovo, the simple epithelium lining the intestine of the chick embryo develops the morphological and biochemical characteristics required for function (*1–4*). The greater part of the epithelium is made up of absorptive cells, but a part differentiates as mucus-producing goblet cells. The latter, barely detectable at 14 days, increase in number and in mucus content during the third week (*2, 4*). That these changes are hormone-dependent is indicated by the failure of goblet cells to exceed the 17-day level in embryos deprived of the hypophysis by removal of the forebrain at 33 hours of incubation (*2, 4*). An increase in goblet cell number in such embryos may be brought about by grafts of the cephalic but not the caudal part of the adenohypophysis (*5*). Since the cephalic region is the only site of adrenocorticotropin and thyrotropin output (*6*), this result may imply that these hormones influence goblet cell production by inducing secretion of glucocorticoid, thyroxine, or both.

The profound effects of early decapitation on the growth and morphogenesis of the intestine (*2*), however, suggest that glucocorticoids or thyroxine may act only as part of a complex system affecting growth and metabolism. This possibility is supported by the fact that whereas exogenous hydrocortisone accelerated the increase of goblet cell number in intact embryos (*7*), neither cortisone nor thyroxine, alone or in combination, tended to normalize epithelial differentiation in decapitated embryos (*2*). We therefore decided to examine goblet cell differentiation and its hormonal control apart from the embryonic environment, by culturing intestinal tissue in a chemically defined medium. Medium 199, which supports intestinal differentiation (*8*), was utilized.

White Leghorn eggs of the Babcock strain, obtained from Ken-Roy Hatchery, Berger, Missouri, were kept in a forced-draft incubator at 37.5°C. At 14 days, the duodenal loop was excised, placed in sterile Ringer solution, and freed from pancreatic tissue and mesentery. The duodenum was then transferred to a petri dish containing culture medium and cut in eight to ten segments 1 to 2 mm in length. Each segment was split open, allowing it to curl into a tube with the luminal surface outside. The fragments obtained from a single duodenum were divided between a control and an experimental culture flask, giving approximately 5 mg of tissue per flask.

Each 25-ml flask contained 2.9 ml of medium 199 (Grand Island Biological Co.), 0.03 ml of penicillin-streptomycin solution (50 unit/ml; Gibco), and 0.1 ml of hormone solvent (control culture) or of hormone solution (experimental culture). Thyroxine solutions were prepared by dissolving 3,3′,5,5′-tetraiodo-DL-thyronine (Sigma) in sterile 0.01*M* NaOH; hydrocortisone 21-sodium succinate (Sigma) was dissolved in sterile 0.9 percent NaCl. Hormone concentrations in the experimental culture flasks were $10^{-9}M$ thyroxine (T4) or $10^{-6}M$ hydrocortisone (HC). After addition of the tissue, the flasks were gassed with a mixture of 95 percent O_2 and 5 percent CO_2, stoppered tightly, and incubated at 38°C. The tissue maintained normal morphology for 48 to 72 hours, as ascertained from paraffin sections of the whole tissue and from electron micrographs of the epithelial cells.

To study the course of goblet cell differentiation in vivo, duodena were collected from embryos at 14 to 19 days of development. Freshly excised duodena were cut into segments and split open as described above. To compensate for possible regional differences in goblet cell numbers, two pieces of each duodenum, from proximal and distal ends, were used. Collected tissue was fixed for 2 to 3 hours in Carnoy fixative, embedded in paraffin, sectioned at 5 μm at right angles to the longitudinal axis, and stained by the periodic acid–Schiff (PAS) procedure.

Up to 17 days, only previllous ridges or primordial villi (*2*) are present, with true villi beginning to grow thereafter. Goblet cells were counted on ridges (or villi) that were sectioned perfectly from tip to base. Counts were made on every third section of a specimen until 100 suitable cross sections of ridges were counted. Results were recorded as total number of goblet cells per 100 ridge (or villus) sections, and also as number confined to the basal halves of the ridges or villi.

Goblet cells increase in vivo from 10 per 100 ridge sections at 14 days to 236 at 18 days (Fig. 1) and 596 at 19 days. The exponential nature of the rise suggests