

One-and-a-half months later, the cultivation was continued on Medium 199 with the addition of 20 per cent bull serum and 0.1 per cent yeast extract. The passages of cells were made at intervals of 2-3 weeks.

The character of cell growth in the examined and control cultures was identical for 2 months, and the cells in these cultures did not differ from one another under the microscope. Infectious virus was not detected in nutrient medium on the twelfth, twenty-eighth and thirty-third days of cultivation by infection of chicks.

On the thirty-fifth day of cultivation we discovered in infected cells the viral antigen with the help of antibodies to RSV-labelled isothiocyanate fluorescein using Coons's method.

We observed very clear fluorescence of cytoplasm of infected cultures and also considerable vacuolization of cytoplasm and enlargement of nucleus in cultures infected by RSV (Fig. 1). We did not observe such a picture in control cultures not infected with RSV.

At the beginning of the third month of cultivation the foci of intensive cell multiplication in the flask were noted in the infected culture.

Fig. 2 demonstrates the foci of multilayered growth appearing during the following days. Gradually, the cell growth became irregular, forming the multilayered cell mass that grew in different directions (Fig. 3). In control cultures we did not observe any signs of transformation; their growth remained monolayered and became very poor (Fig. 4).

The same results were obtained by Koprowski, Jensen and Girardi (personal communication).

The afore-stated character of transformation gives some evidence to suppose the malignization of our cultures, but further experiments are needed to draw a definite conclusion.

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Acidophilia of Aortic Elastin in the Child

In a previous note¹ some results were presented to show that if 5-7- μ sections of human aorta were exposed to 0.025 per cent bromphenol blue (B.D.H. indicator) in pH 8.2 Sorensen buffer, then left in distilled or ordinary tap water, aortæ from young subjects retained the dye in the elastic laminae considerably better than those from the old.

As the maximum resistance to leaching appeared in the young child, a further series has been collected, concentrating on this particular age group. In all, 259 aortæ of children under 7 and 261 aortæ of older individuals, a total of 520, have now been examined. In each case, a portion of descending thoracic aorta was fixed in 10 per cent formol-saline buffered with chalk, embedded in paraffin and cut at 5-7 μ .

The method of reading the results has been simplified, and is now more accurate and reproducible. We stain for 20 min in the dye solution, leave overnight (20 h) in water, and grade the residual laminar colour in water under the low power of the microscope: no colour = 0, detectable colour = +, definite colour = ++, strong

colour = +++ . The overnight reading seems to be the most consistent as readings at shorter intervals are more affected by minor variations in technique.

In aortæ from subjects older than 7 years, the bromphenol blue is invariably leached by overnight immersion in water. Up to 7, resistance of the dye to leaching develops (Table 1) and regresses (Table 2).

Table 1. ANALYSIS OF 184 AORTÆ UNDER 1 MONTH SOMATIC AGE

Weight of infant in grams	Aortæ arranged in groups according to laminar colour remaining after overnight leaching in water			
	0	+	++	+++
60-500	5	10	1	—
500-1,000	5	19	3	4
1,000-2,000	4	14	11	16
2,000-5,000	2	9	13	68

Table 2. ANALYSIS OF 336 AORTÆ FROM SUBJECTS AGED 1 MONTH TO 95 YEARS

Somatic age of subject in years	Aortæ arranged in groups according to laminar colour remaining after overnight leaching in water			
	0	+	++	+++
1/12-2	—	—	—	59
2-3	—	—	1	2
3-4	—	5	—	2
4-7	2	4	—	—
7-20	17	—	—	—
20-95	244	—	—	—

The results may be summarized as follows. During gestation and the neonatal period, bromphenol blue retention in aortic laminae is roughly proportional to the weight of the subject, reaching a maximum level at about the age of one month. That level is retained until the age of 2 years, when it begins to fall. There is little left at 4 years, and the maximum somatic age which has shown any degree of bromphenol blue retention is 6 years 10 months.

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Metabolic Activity of Neural Crest Tumours in Tissue Culture

NUMEROUS reports have described the raised urinary excretion of metabolites of norepinephrine, and its precursors, by children with tumours of neural crest origin¹⁻⁷. It has also been observed that many of these patients show increased norepinephrine production but produce little or no physiological evidence of increased secretion of this potent catecholamine. Some possible explanations for this apparent paradox are as follows: (1) There might be an adaptation to increased blood-levels of norepinephrine. (2) The norepinephrine might be inactivated within the tumour before release into the blood. (3) A combination of the foregoing two mechanisms might be acting. (4) The rate of inactivation of norepinephrine in tissues other than the tumour (liver, kidney, etc.) might be increased. In a previous investigation, La Brosse and Karon have presented evidence that neural crest tumours contain catechol-O-methyltransferase (COMT) activity⁸. Since the 3-O-methylated product of norepinephrine has been shown by Evarts *et al.*⁹ to be physiologically inactive in comparison with norepinephrine, the finding that COMT activity existed within the tumour indicated that, at least in some cases, norepinephrine may be inactivated before being released into the blood.