Table 2. Discriminant function coefficients for comparison of excretion patterns in cancer with those in matched normal controls. Coefficients are in standard units, hence comparable within each comparison column.

Variate	Breast cancer premenopause (N = 7) compared with premenopause normal (N = 24)	Breast cancer postmenopause (N = 21) compared with postmenopause normal (N = 20)	Prostate cancer (N = 21) compared with normal men (N = 39)
A. 18-variate discriminant		*Baseline measurements*	
Age	3.8	0.3	0.2
Weight	−6.5	−1.4	−1.3
Height	−1.3	1.0	−0.0
Specific gravity	5.2	−0.2	−0.8
Creatinine	6.6	1.1	−0.1
		Androgen complex	
Androsterone	−9.5	−3.8	−1.5
Etiocholanolone	0.3	1.6	−0.7
11-oxygenated ketosteroids	−5.1	−0.7	0.9
Beta fraction	4.5	−1.0	1.2
Pregnanediol	−5.7	2.3	1.3
		Estrogen complex	
Estrone	−7.1	−0.1	−2.4
Estradiol	3.0	−2.1	−1.0
Estriol	5.6	2.9	2.1
		Corticoid complex	
Porter-Silber corticoids	1.6	2.4	0.3
17-Ketogenic steroids	0.6	−0.2	0.1
		Gonadotropin residue complex	
Gonadotropin residue:			
Biological activity	0.0	0.4	−1.8
Chemical composition			
Protein	5.4	2.6	4.7
Carbohydrate (protein-bound)	8.0	−1.5	−1.4
Determination (R^2)	0.91	0.69	0.75
P	<0.010	<0.025	<0.001
B. 3-variate discriminant			
Androsterone	−2.7	−0.9	−1.5
Gonadotropin residue protein	2.0	1.1	1.9
Estriol	0.7	1.6	0.3
Determination (R^2)	0.63	0.47	0.56
P	<0.001	<0.001	<0.001

cancer and normal subjects. This analysis, however, is not primarily aimed at possible use of the discriminant in diagnosis, but at description of the pattern of endocrine disturbance related to the presence of cancer. Indeed, although this same endocrine disturbance is not found in sick (nontumor) controls, there is indication of a complex endocrine disturbance associated with benign tumors of the breast and of the prostate.

Although the origin and endocrine activity, if any, of the protein fraction of the gonadotropin residue is not known, increased protein was associated with a multiple steroid disturbance in the cancer patients. The physiological significance of this combination of abnormalities in the excretion pattern must await further investigation, but the result suggests a more extensive disturbance of hormone relationships in cancer than hitherto reported. This multiple disturbance in both breast and prostate cancer may be related, among other possibilities, to their similar cell type as adenocarcinomas, that is, originating in columnar epithelium, or to the analogous response of breast and prostate tissue as target organs of the endocrine glands.

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Atrophy of Skeletal Muscle in Chick Embryos Treated with Botulinum Toxin

Abstract. *Botulinum toxin was given in large intravenous doses to 7- and 12-day chick embryos. Atrophy of skeletal muscle resulted without significant atrophy of other organs. The histological appearance of muscle was consistent with denervation. The results suggest that neural acetylcholine release may play a significant role in "trophic transmission" from nerve to muscle.*

Botulinum toxin is known to prevent the release of acetylcholine from motor nerve terminals (*1*). Local injection of the toxin reproduces certain physiological effects of muscle denervation (motor paralysis, spread of the receptor zone, and spontaneous fibrillations) (*2*). This suggests that acetylcholine may serve a dual function, both as an impulse transmitter and as a "trophic transmitter" to muscle. Experimental interruption of axonal conduction by local anesthetics (*3*) and by nerve compression (*4*) produces motor paralysis, but fails to eliminate the nerve's trophic effect. The possibility remains that the spontaneous quantal release of acetylcholine by the nerve terminals (*5*) is adequate to prevent the atrophic consequences of denervation.

A structural change has been sought in the motor end plates of frog and cat muscles injected locally with botulinum toxin, but none was found with electron microscopic techniques (*2*). Feng *et al.* (*6*) observed hypertrophy of "slow" adult fowl muscle, with shrinkage of "fast" muscle, up to 8 weeks after local injection of botulinum toxin.

I have administered comparatively enormous amounts of botulinum toxin intravenously to chick embryos, with resulting atrophy and degeneration of the skeletal muscle fibers. The nature of the atrophy suggests true denervation, a point which will be defined by further studies. Systemic administration of botulinum toxin in minute amounts produces death by respiratory paralysis in most animals; since respiratory gas exchange in the chick embryo is accomplished by passive diffusion across the chorioallantoic membrane (*7*), the embryo readily survives doses which would be lethal to 20,000 or more hatched chickens (*8*).

The method of intravenous chorioallantoic injection was described previously (*9*). Type A crystalline botu-

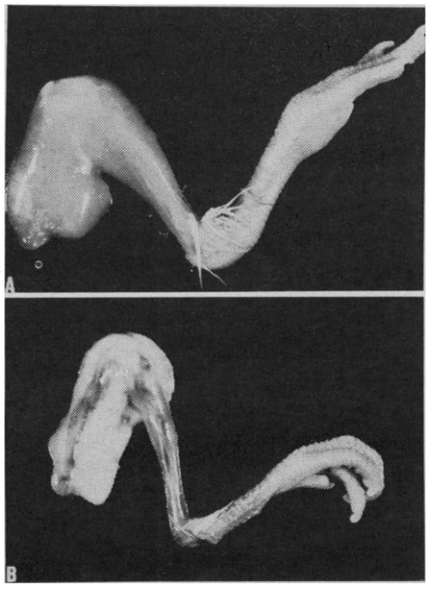

Fig. 1. Thigh and leg from (*A*) a normal 19-day chick embryo and (*B*) a 19-day chick embryo injected with botulinum toxin on the 7th and 9th days of incubation.

linum toxin (*10*), stored at 3°C in acetate buffer, was freshly diluted in chick embryo Ringer's solution (*11*) immediately before use. Ten embryos (group A) were injected with 0.1 ml of solution containing 39 μg of botulinum toxin on the 7th day of incubation, and again 48 hours later. Fourteen embryos (group B) were given a single 0.1-ml injection containing 30 μg, on the 12th day of incubation. Control embryos in each age group received 0.1 ml of Ringer's solution. They were incubated at 37.7°C in a humidified, forced-draft incubator. All embryos were killed on the 19th day of incubation. The yolk sacs and extraembryonic membranes were removed from the embryos which were then washed, blotted, and weighed. One thigh and leg with all attached muscles was dissected from each embryo, and was skinned, blotted, and weighed. After all muscle tissue was removed, the femur, tibia, and fibula were weighed; the difference between limb weight and bone weight was taken as the weight of skeletal muscle. The opposite leg of each embryo was fixed in 10 percent formalin with calcium chloride, for histological or histochemical processing. The heart and liver were dissected out whole, blotted, and weighed. Three hearts were fixed in formalin for histological examination.

Table 1. The mean weights of the body, lower limb muscles, heart, and liver, of chick embryos injected with botulinum toxin on the 7th and 9th days of incubation (group A); chick embryos injected with toxin on the 12th day of incubation (group B); and control embryos. All embryos were killed on the 19th day of incubation.

Group	Mean wt. (g)	S. E.	Reduction in wt. (%)
	Body		
Control	27.4	1.20	
Group A	14.31	0.536	47.7
Group B	15.70	0.581	42.7
	Limb muscle		
Control	1.37	0.053	
Group A	0.204	0.007	85
Group B	0.324	0.026	76.4
	Heart		
Control	0.175	0.041	
Group A	0.130	0.007	25.7
Group B	0.126	0.007	28.0
	Liver		
Control	0.562	0.045	
Group A	0.475	0.039	15.5
Group B	0.461	0.127	17.9

The embryos injected with botulinum toxin were smaller and lighter in weight than the controls, but otherwise had matured normally for their age (*12*). Externally they showed severe ankylosis of multiple joints, a condition known as arthrogryposis multiplex congenita, which results from skeletal immobilization during embryonic development (*13*). Apart from the additional finding of a slightly shortened upper beak, the abnormalities were limited to the muscular system. On gross observation, all skeletal muscles of the body and limbs were strikingly shrunken and fatty (Fig. 1). This appearance was most marked in the embryo injected with toxin on the 7th day of incubation.

The weights of lower limb muscle and of other organs are presented in Table 1. The reduction in muscle weight was 76.4 to 85 percent, in contrast to a reduction of only 15.5 to 17.9 percent for the liver. Since the growth rate of the liver and lower limb are normally parallel between the 7th and 19th days of incubation (*7*), it is clear that a "generally retarding" process cannot account for the findings. The reduction in heart weight was likewise far less than that of skeletal muscle, and the cardiac muscle appeared histologically normal in the experimental chicks. This indicates that botulinum does not exert a "general toxic effect" on striated muscle.

The reduction of skeletal muscle weight was significantly greater in group A than in group B ($p<.005$), while there was no significant difference in the heart and liver weights of the two groups. The fact that more prolonged treatment adversely affected skeletal muscle, but not the other organs, adds further evidence in favor of a specific action of botulinum toxin on skeletal muscle.

Histologically, the muscles of embryos injected with toxin on the 12th day of incubation showed the following features (Fig. 2): reduction in muscle bulk, in transverse and longitudinal sections; reduction in individual fiber diameter in transverse sections; increase in number of sarcolemmal nuclei; rounding of the contours of sarcolemmal nuclei, and clumping of groups of nuclei; interstitial fatty infiltration, and large deposits of fat; phagocytosis of degenerated muscle fibers; increased endomysial fibrous tissue; small spindle-shaped cells distributed interstitially in the more severely affected regions.

The degenerative and fatty changes were more advanced in muscles of embryos injected with toxin on the 7th day. Often only a few strands of degenerated muscle could be identified in a sea of fat. In many, but not all, of the limbs from treated chicks, some

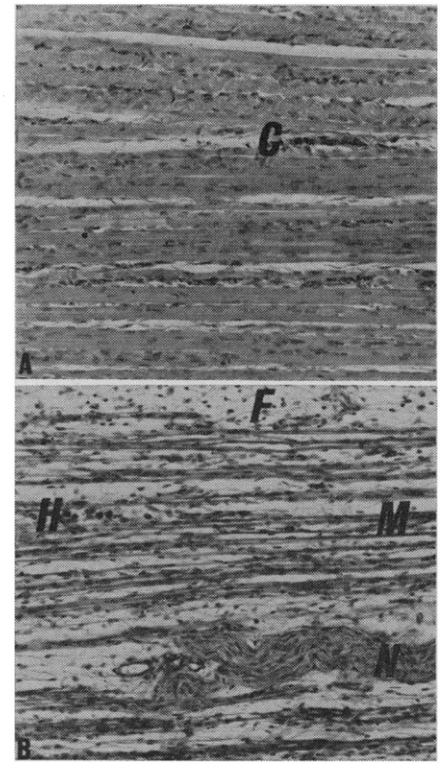

Fig. 2. (*A*) Section through the quadriceps muscle of a normal chick embryo after 19 days of incubation (× 200). A capillary is labeled *C*. (*B*) The same muscle from a 19-day chick embryo injected with botulinum toxin on the 12th day of incubation, showing the drastically reduced size of the muscle fibers (*M*), the infiltrating fat cells (*F*), the presence of an intramuscular nerve (*N*), and phagocytic histocytes (*H*). There is also a moderate increase in the number of sarcolemmal nuclei. (× 200)

normal-sized fibers scattered singly, or in groups, were found. These fibers retained a primitive appearance with a myotubal structure and centrally placed nuclei. Intramuscular nerve bundles were prominent (when stained with hematoxylin and eosin), but further examinations will be necessary to evaluate the structural integrity of the axones and nerve terminals.

These histological findings are consistent with denervation atrophy and degeneration (*14*), but occur on a greatly accelerated scale in the chick embryo, as compared with mature animals. Although skeletal muscles are already innervated in embryos of 7 days' incubation (*15*), such as were used in the present experiments, a similar picture has been described for chick embryo limb muscles which have never received primary innervation (*16*).

Theoretically, one cannot distinguish with absolute certainty between denervation atrophy and disuse atrophy of muscle by purely morphological criteria (*17*). However, the rapid appearance of atrophy, fat accumulation, and subsequent fiber degeneration favor denervation atrophy. In the present experiments, atrophy and fat accumulation were well advanced by 1 week after the injection of toxin, strongly suggesting the effects of denervation. The presence of even a few large but immature muscle fibers remains problematical.

These results are offered as preliminary evidence favoring a "trophic" influence of neural acetylcholine release on skeletal muscle. Further studies with the chick embryo confirming the specific physiological action of botulinum toxin, and outlining the pattern of disuse atrophy, are needed to support this hypothesis. Undoubtedly, botulinum toxin will prove to be a powerful tool for exploring the trophic role of acetylcholine release on other aspects of embryonic development.

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Electroencephalographic Correlogram Ratios and Their Stability

Abstract. *Autocorrelations of electroencephalograms can be reduced to ratios of estimated power among distinguishable parameters of typical tracings: the dominant rhythm, background activity, abundance, and total power. These data reduction methods permit statistical evaluation of differences among experimental conditions, thus extending the usefulness of graphic correlograms in research. Ratios discriminate between two experimental conditions and two subjects, while showing stability over days.*

The potential usefulness of correlation analysis to research in electroencephalography has been described in several reports (*1*). In the few studies where it has been used, experimenters have typically depended upon visual inspection of the correlogram, counted dominant wave frequency, or measured phase shifts. It is proposed that additional data can be extracted from correlograms, can be reduced to terms which are meaningful conceptually and which are quantified approximations of significant parameters, and can be subjected to statistical analysis for the testing of hypotheses.

Inspection of a typical cyclic correlogram (Fig. 1) shows most obviously the period of the dominant wave pattern. (However, its precise determination cannot be made from the first cycle because wave length may be distorted in this region.) In addition, the display contains displacement, which is a function mainly of low-frequency waves and non-rhythmic components, identifiable as a lack of balance around zero voltage at zero time delay and restoration of balance through time thereafter. A third feature is decay in amplitude of successive cycles which is attributable to the fact that the electroencephalogram (EEG) has a continuous (or non-line) power spectrum with characteristics related to amplitude and phase modulation.

According to current theory, the EEG rhythm "represents massed synaptic potentials of apical dendrites of mainly pyramidal cells becoming synchronous and oscillating as fields of maximal amplitudes," and infant patterns develop into "more rigidly defined synchronized alpha rhythms of adult life" (*2*). In line with this concept, the term "dominant synchronized rhythm" (DSR) shall be used to refer to the typical cyclic pattern reflected in the correlogram. Since this dominant rhythm resembles a modulated sine wave of random amplitude and phase, and desynchronization of the EEG leaves a pattern resembling limited band-width noise, then the mathematical demonstrations of correlogram interpretation (*3*) are appropriate for a first approximation analysis. (i) A modulated sine wave of random amplitude and phase yields a cosine correlogram with an envelope which decays exponentially. (ii) Restricted bandwidth noise (with no dominant frequency) gives an exponential correlogram. (iii) The correlogram of mixed wave forms is equivalent to the sum of correlograms of the separate wave forms. For the analysis in this report, the exponential functions are to be treated as linear.

Figure 1 is a diagram of a typical EEG autocorrelation function and of the basis for quantification. Vertical axes *AG*, *BH*, and *CI* identify the decaying correlogram cyclic amplitude at the zero-, half-, and full-period points respectively. Displacement is represented by the line connecting the midpoints of the axes (points *DEF*).

Values to be used for analysis may be taken directly from the graphic display. The ordinate value of point *A* is a good estimate of the mean power in the EEG epoch analyzed, or its mean