identified the sample by noting perceptual experience which was in agreement with that which was predicted solely on the basis of the composition of the sample in comparison with the results of the familiarization training. In the one case in which this was not so, the response was almost immediately changed by the subject. However, as this response change occurred after the 10-second deadline, the response was not scored as an agreement. The null hypothesis can be rejected with a P value considerably beyond the .01 value.

A further test was made with 12 subjects who had no previous taste training experience, had never tasted mannose before, and who had no knowledge of the experiment nor any intimation of the results expected. The same taste testing procedure was followed as outlined above, except that each subject was asked to tell in his own words the nature of the taste experience. Each subject was given only one test.

In the 12 tests, 10 responses were in agreement with predictions based on the composition of the administered sample ($P = .019$ one-tailed). One subject reported the equilibrium mixture as bitter only, while another stated that the α-anomer was initially sweet, but started to change "somewhat" within a few seconds and became "unpleasant" about 1 or 2 *minutes* later. (It should be noted that the taste threshold for the α-anomer appears to be significantly higher than that of the β-anomer; this may account in itself for the first disagreement. That is, the mild taste of the α-anomer was not perceived by this subject. In the second disagreement, the relatively long time that elapsed before the "unpleasant" report leads one to surmise that sufficient mutarotation may have occurred so that sufficient β-anomer may have been produced to stimulate an "unpleasant" response.)

It would appear that the extremely slight difference in molecular structure between the α- and β-anomers of D-mannose is sufficient to cause a rather dramatic difference in the taste of these substances. [It is interesting to note that in a similar but more complicated case of a disaccharide, 6-α-D-glucopyranosyl-D-glucose is sweet but its anomer, 6-β-D-glucopyranosyl-D-glucose, is bitter (*7*). Furthermore, Pangborn and Gee (*8*) have shown that the α-anomers of the monosaccharides D-glucose and D-galactose are sweeter than the β-anomers of these compounds.] It is apparent that a high degree of physicochemical stereospecificity must be exhibited by taste receptors.

This work is being continued by efforts to separate sufficient pure β-D-mannose so that a direct comparison of the tastes of these two anomers can be made (*9*).

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6. The sample was administered in the solid state to obtain as intense a taste stimulation as possible. Through preliminary experiments it had been established that solution concentration does not appear to be a relevant variable in this case, the only effect of concentration being that of altering the intensity of taste. The effect of mutarotation in the α-anomer makes administration of the sample as the aqueous solution extremely inconvenient, as a new sample must be prepared rapidly before each individual test; sufficient β-anomer to affect the taste is developed within a few minutes after the preparation of an aqueous α-anomer solution.
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Decrease of Acetylcholinesterase Activity along Peripheral Nerves

Abstract. Acetylcholinesterase activity was examined in segments of some peripheral nerves anatomically accessible throughout their length. It decreases linearly with distance from cell bodies, the activity of the terminal part of the nerve being about 30 to 40 percent lower than that of its most proximal part.

Although electrophysiological and morphological features of nerve fibers are constant throughout the length of unbranched parts of axons, evidence is accumulating that the biochemical organization of axons fails to exhibit similar uniformity. Gradients of composition and metabolic activity have been described in normal peripheral nerves (*1*, *2*). Indirect evidence of changes of biochemical properties along axons is provided by many peripheral neuropathies, in which the distal parts of nerves appear to be more vulnerable to circulating toxic agents than the proximal parts.

If a substance is produced in the perikaryon and metabolized in the cell processes, it has to be transferred from one site to the other. For these substances the axon, besides being the possible seat of metabolism, constitutes a traffic line. At each level of the axon the concentration of a substance originating from the perikaryon is determined by the rates of arrival, of utilization, and of transport to more distal portions of the axon. The distribution of the substance in the length of the axon would thus provide some information about its fate in the neuron.

With this in view we investigated the distribution of acetylcholinesterase in the peripheral nerves. The known physiological action of this enzyme occurs at the nerve endings, and there are manifold indications (*3*, *4*) that, like many other enzymes (*5*), it is synthesized in the perikarya.

In view of large differences in enzyme activity of individual neurons (*6*), the assembly of the same fibers, without admixture of bundles which join or leave the nerve at various levels, should be analyzed throughout. Moreover, all fibers should be approximately equally long and accessible throughout most of their length. In these respects most nerves to extremities are inconvenient for an analysis of biochemical gradients. In a preliminary note (*2*) we described a proximo-distal gradient of total cholinesterase activity in the phrenic nerve of dog in which these difficulties are relatively small.

In the present experiments acetylcholinesterase activity, which provides a clearer picture of neuronal distribution, was determined at various levels of the phrenic and the hypoglossal nerves and of the nerve to the levator scapulae, originating at C_5. These nerves run without anastomoses and either have fibers of fairly uniform length or may be rendered homogeneous in this respect by rejection of separable fascicles shorter than the

main bundle. A few other nerves, accessible separately only in their distal parts, were also analyzed.

Total length of nerves was defined, somewhat arbitrarily, as the distance from the emergence from vertebrae up to about 1 cm above the entry of the nerve into the muscle. Thus, nerve roots and intramuscular twigs were omitted.

The nerves were taken from dogs under barbiturate anesthesia, the epineurium was removed under the dissecting microscope, and in multifascicular nerves, the integrity of all bundles to be used for analysis was carefully checked throughout the length of the nerve. Then the nerve was divided into four or five consecutive segments, and the length of each was measured.

Determinations of acetylcholinesterase activity were made on homogenates by a micromodification (*7*) of the iodometric method (*8*). Acetylthiocholine was used as substrate; pseudocholinesterases were inhibited by diisopropyl fluorophosphate (10^{-8} *M*) (*9*). Total protein content was determined in the same sample.

In all investigated nerves, acetylcholinesterase activity decreases with distance from cell bodies. Figure 1 shows mean values of acetylcholinesterase activity at various levels of each kind of nerve plotted against the relative distance from the beginning of the nerve.

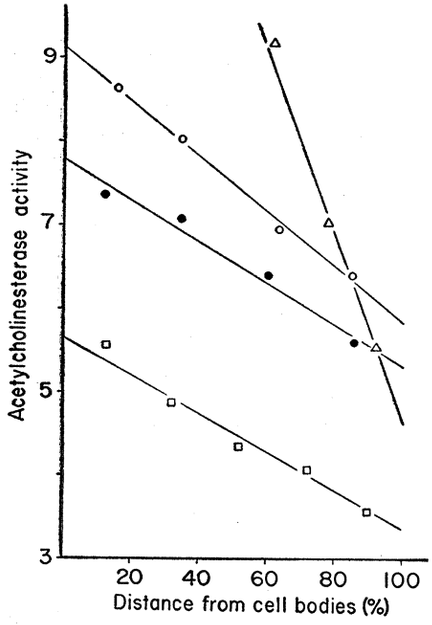

Fig. 1. Mean values of acetylcholinesterase activity (in micromoles of acetylthiocholine split by 10 mg of nerve proteins at 37°C in 2 hours) in peripheral nerves plotted against distance from cell bodies. Calculated regression lines are also shown. Squares, phrenic nerve; dots, hypoglossal nerve; circles, nerve to levator scapulae; triangles, sural nerve.

Table 1. Differences in acetylcholinesterase (AChE) activity between the most proximal and the most distal part of nerves, in percentage of activity of the proximal part. In branches of the tibial nerve the difference shown is that between the beginning and the end of the anatomically separable bundles. Numbers in parentheses were obtained by extrapolation of calculated regression lines towards the beginning of axons and show the probable drop of enzyme activity throughout their length.

Nerve	Number of nerves	Segments in each nerve (No.)	Average nerve length (mm)	Fraction of axonal length analyzed	Decrease of AChE between beginning and end of nerve (%)	Test for linearity of regression	
						F	$F_{0.90}$
Phrenic	10	5	260	Whole*	40	1.47	8.59
Hypoglossal	10	4	73	Whole*	31	1.56	9.46
Levator scapulae	6	4	106	Whole*	36	3.18	9.42
Sural	8	3	272	Distal third	46 (69)	3.06	61.74
Branches to shank muscles	6	3	223	Distal fifth	26 (65)	4.54	61.22
Branches to hamstring muscles	1	3	116	Distal half	37 (53)		

* As defined in the text.

In Table 1 the differences in activity between the most proximal and the most distal part of the nerve are expressed as percentages of activity in the proximal part. In nerves anatomically accessible over their entire length, the decrease is linear throughout, or more precisely, the hypothesis of linearity tested by linearity tests (*10*) is far from being rejected.

The trend appears to be similar in nerves available only in their distal parts, although it is not certain whether the observed relationship can be extrapolated to the proximal parts of the axons of those nerves. The calculated drop of activity over the total length of these axons is shown in parentheses in the column in which experimental values for the directly analyzed fraction of axonal length are given.

There is a suggestion that in long axons the decline is larger than in shorter axons. The decrease may exceed 60 percent in various branches of the tibial nerve as contrasted with less than 40 percent in the hypoglossal nerve and the nerve to levator scapulae. The drop in the phrenic nerve, however, is not consistent with this suggestion, and it shows that other factors, besides axonal length, may influence the gradient of acetylcholinesterase activity in the nerve.

The topographical distribution of acetylcholinesterase along axons does not seem to have been investigated systematically before, except in embryological studies (*4, 11*). The existence of gradients is, however, suggested by certain published data. Thus, in Nachmansohn's table (*12*) showing acetylcholinesterase activity in various parts of the nervous system of the dog, the activity of the sciatic is lower than that of either ventral or dorsal lumbar nerve roots.

If the hypothesis that this enzyme is synthesized exclusively in the perikaryon is correct (see also *13*), the simplest interpretation of our results is that a considerable fraction of acetylcholinesterase produced by the neuron is in some way used up in the axonal path before it reaches the nerve endings. The linear decrease would indicate that a constant amount of the enzyme is normally metabolized by unit length of a given nerve. Other possibilities cannot, however, be ruled out. The difference in enzyme activity between the proximal and the distal part of the axon might have been built up during ontogenesis and persist into the adult life, or rate of transport may vary along the nerve and create the observed differences. Too little is known about the mechanism of intra-axonal transport to determine which of these possibilities is correct (*14*).

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Extraction of Distinctive Antigens from Neoplastic Tissue

Abstract. Antigens have been obtained from HeLa and J111 cells by treatment with fluorocarbon. Cross absorption studies revealed that the antigen from HeLa cells had no serologic relationship to normal human uterus. Antibody for HeLa extract coated red blood cells was found in 25 percent of the sera from patients with malignant diseases.

Taylor *et al.* (*1*) and DeCarvalho (*2*) reported that antigens were obtained from both fresh tumor tissues and tumor cell lines of human and chicken origin by treatment of extracts with fluorocarbon. These antigens were specific for the tumor of origin when assayed by complement-fixation and gel-diffusion against immune rabbit sera. Neither chick muscle, primary monkey kidney cells, nor primary human amnion cells contained similar antigenic material.

This report describes the finding of antibody to HeLa cell extracts treated with Genetron, in the sera of human beings with malignant metastatic disease.

HeLa cells and J111 cells grown in medium 199 supplemented with 10 to 15 percent normal calf serum were used as the source of antigenic material. Uterine tissue was obtained from a normal human uterus freshly removed. These tissues were homogenized at 4°C for 5 minutes at high speed in a Vir-Tis 45 homogenizer in distilled water to yield 20-percent suspensions. The aqueous extracts were centrifuged at 3000*g* at 4°C for 10 minutes and the pellets were discarded. These extracts were designated as crude antigens (C). Rabbits were immunized with a total of 6 ml given in six intravenous injections.

One-half volume of Genetron 113 was added to each of the crude extracts and mixed for 1 minute at 4°C at high speed in a Vir-Tis 45 homogenizer. Each Genetron-treated extract was centrifuged at 3000*g* at 4°C for 10 minutes and the supernatant was saved. This process was repeated with the supernatant fluids for a total of five extractions. This procedure apparently removes nonspecific protein, leaving a specific antigen in the aqueous phase. Only the HeLa and J111 extracts contained material which was precipitable with trichloroacetic acid. Rabbits were given a series of six intravenous injections of the extracts, equal to a total of 250 μg of protein nitrogen. Other rabbits were injected with a like volume of the final extract of normal uterus even though no protein was present. These extracts were designated as Genetron antigens (G) (*3*).

The diluent for the complement-fixation test was 0.85 percent saline. The hemolytic system was composed of 0.3 ml of complement containing two full units and 0.6 ml of 1 percent optimally sensitized sheep red cells. Optimal dilutions of antigens were determined by box titrations, and sera were titrated with these optimal dilutions. Titers are expressed as the reciprocal of the highest dilution of serum showing 100-percent fixation.

Table 1 shows the magnitude of cross reactivity among the crude antigens (C) and their antisera (C) as determined by complement-fixation. The cross reactions were virtually eliminated by extraction with Genetron. It is noteworthy that all antigenicity was lost from uterus (G antigens and antisera). These data are in agreement with the findings of Coriell *et al.* that common antigens are present in long-term tissue-culture cell lines (*4*). The presence of common antigens was also observed in the crude uterus extract. While the results given in Table 1 are confined to data obtained by using a normal human uterus, similar results were obtained with normal human skin, muscle, and liver.

Table 1. Specificity of cell extracts obtained by Genetron treatment. Symbols: C, crude antigens and their antisera; G, Genetron antigens and their antisera.

Antigen	Complement-fixation titers with antisera					
	HeLa(C)	J111(C)	Uterus(C)	HeLa(G)	J111(G)	Uterus(G)
HeLa(C)	320	80	80	640	<5	<5
J111(C)	40	80	40	<5	80	<5
Uterus(C)	40	80	320	<5	<5	<5
HeLa(G)	320	10	<5	1280	10	<5
J111(G)	<5	320	<5	<5	640	<5
Uterus(G)	<5	<5	<5	<5	<5	<5

Table 2. Specificity of antisera as determined by absorption.

Antigen used for immunization	Absorption of antiserum	Antiserum titer*	
		Uterus (C)	HeLa (G)
Uterus(C)	None	320	<5
Uterus(C)	Uterus(C)	<5	<5
Uterus(C)	HeLa(C)	10	<5
Uterus(C)	HeLa(G)	320	<5
HeLa(G)	None	<5	640
HeLa(G)	HeLa(G)	<5	10
HeLa(G)	HeLa(C)	<5	40
HeLa(G)	Uterus(C)	<5	640

*Determined by complement-fixation.

Antisera to the crude uterus extract and to the Genetron HeLa extract were absorbed as indicated in Table 2. Since the Genetron-treated HeLa extract was not particulate, it was added to each antiserum in equal volumes, incubated at 4°C overnight, and centrifuged at 30,000*g* for 30 minutes. Each absorbed serum was titered against an optimal dilution of each antigen used for immunization. It is evident that an antigen is present in HeLa cells which is serologically distinct from antigens in normal uterus.

Using the tanned red cell hemagglutination technique as modified by McKenna (*5*), we sensitized red cells with the product obtained by Genetron treatment of HeLa cells, or with normal human uterus treated in the same way. A total of 366 human sera have been assayed by this technique (*6*). Of the 193 sera from patients with various malignancies, 49 (25 percent) showed evidence of antibody capable of reacting with the HeLa extract, and nine (5 percent) other sera reacted with the J111 extract. None of the sera from patients with malignancy reacted with the uterine material and none of the remaining 173 sera from patients without malignancy reacted with extracts of HeLa, J111, or uterus. Of 49 patients